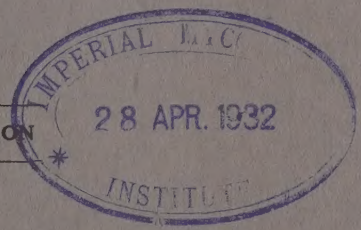
Technical Bulletin No. 118 January, 1932

INVESTIGATIONS IN THE MOSAIC DISEASE OF BEAN

(*Phaseolus vulgaris L.*)



RAY NELSON

AGRICULTURAL EXPERIMENT STATION

MICHIGAN STATE COLLEGE
Of Agriculture and Applied Science

SECTION OF BOTANY

East Lansing, Michigan

TABLE OF CONTENTS

	Page
INTRODUCTION	3
TYPICAL BEAN MOSAIC	
THE DISEASE	5
History and Distribution	5
Economic Aspects	7
Symptoms	8
Transmission	
By sap expressed from diseased plants	13
By insects	13
By seed	15
Vascular anatomy and seed infection	23
Other methods of transmission	25
Varietal, Specific, and Generic Susceptibility	25
CYTOLOGICAL INVESTIGATIONS	27
Review and discussion of literature of special significance in the present investigation	28
Material and methods of the cytological investigation	29
Comparative observations on sections from normal and mosaic plants	30
The normal chloroplast and its inclusions	31
Chloroplast changes in mosaic plants	32
The coccoid bodies in other tissues	35
BACTERIOLOGICAL INVESTIGATIONS	36
Review and discussion of literature	36
Material and methods of the bacteriological investigation	39
Preparation of media	39
Material for the isolation experiments	41
Technic employed in making isolations	41
Results of the isolation experiments	43
RUGOSE MOSAIC	50
History and Description of Symptoms	50
Bacteriological Investigations	51
Cytological Observations	54
PATHOGENESIS	56
DISCUSSION	56
SUMMARY	60
ACKNOWLEDGEMENTS	63
BIBLIOGRAPHY	63
EXPLANATION OF PLATES	68
PLATES	73

INVESTIGATIONS IN THE MOSAIC DISEASE OF BEAN

(*Phaseolus vulgaris L.*)[1]

RAY NELSON

INTRODUCTION

In 1886[2] Mayer described and proved the infectious nature of a disease of tobacco which he designated as "Mosaikkrankheit." Six years later, Iwanowski (1894) showed that the causal agent was present in the sap from diseased plants after it had been passed through porcelain bacterial filters. This discovery was an epochal contribution to the scientific knowledge of the etiology of disease. To the previously known kinds of pathogens was now added a more alluring one, the submicroscopic, filterable virus. Beijerinck (1899) confirmed the results obtained by Iwanowski on the filterability of the virus and believed that the nature of the causal agent could best be explained on the assumption that it existed as a "contagium vivum fluidum." Although Beijerinck's conception of the virus as a living fluid endowed with the capacity for reproducing itself is no longer tenable in the light of more recent filtration experiments, the real nature of the virus is still enshrouded in mystery.

The literature dealing with investigations in plant virus diseases has increased enormously during the past decade. In general, the investigations have followed two lines, those which have facilitated separation and classification of the diseases on various hosts, and cytological studies which have attempted by microscopical methods to determine the nature of the causal agents. No marked success has followed the attempts to demonstrate organisms in the tissues of diseased plants, but the cytological studies have revealed the presence, in some plants, of cell inclusions which seem to be comparable to the inclusions that characterize certain animal virus diseases. The failure to demonstrate organisms consistently in the tissues of plants affected with some of the more common virus diseases has probably deterred, to a certain extent, investigations in the bacteriology of these diseases. It is certain that this field of research has attracted comparatively few workers, and all of the investigations have largely followed a single method of attack. The application of new methods to the study of the possibility of the viruses being filterable forms of bacteria has not yet been tested. The pressing need for knowledge concerning the actual nature of the viruses and the many obscure and uncontrollable factors which hinder the investigations in these diseases, because of this unknown etiology, should actuate a desire to

[1]A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the department of Botany, University of Michigan.

[2]Reference is made by date to "Bibliography" page 63.

test thoroughly every possible method that may aid in the solution of this perplexing problem.

Despite the apparent failure to culture organisms from the tissues of plants affected with virus diseases, sufficient evidence has not been accumulated to show that this is an impossibility. Although calibration experiments have provided evidence which indicates that the virus of tobacco mosaic exists in a minimum size that is beyond the range of microscopic resolution, no proof has been offered to show that this minimum size characterizes the virus in all of its stages. Filtration experiments, on the contrary, have established the fact that passage through filters decreases the potency of the sap from diseased plants, indicating perhaps a removal of larger particles than those that traverse the pores of the filters. It would be strange indeed if the virus particles of any disease were uniform in size, as such a uniformity does not characterize any known kinds of organisms. The filtration experiments have clearly established the particulate nature of the viruses which, in general, react very similarly to the bacteria.

The existence of filterable stages in the ontogenetic development of bacterial organisms is a revolutionary discovery in the field of bacteriology. While the evidence is unconvincing to many, there seems little doubt that bacteria do exist in filterable forms (Hauduroy, 1929; Hadley, 1931). This discovery has opened up new horizons in bacteriology, and, undoubtedly, from it will come a great stimulus to the study of disease, particularly those diseases of animals and plants caused by the filterable viruses. The recent results obtained by Swezy and Severin (1930) in their bacteriological studies of the curly top disease of the sugar beet indicate that filterable forms of a bacterial organism are present in both the best root and in the leafhopper that transmits the disease. Confirmation of these results will be awaited with interest, because the causal agent of the curly top disease from either the best or from the insect vector has definitely been proved to be a filterable organism.

This paper is a presentation of the results that have been obtained in studies of bean mosaic carried on, for the most part, at Michigan State College, but including also work done at the University of Michigan in 1927-28. The cytological and bacteriological investigations are the subsequent results of some observations made during the winter of 1927. At that time, while examining sections of living tissue from mosaic bean plants, some observations were made which indicated that organisms were associated with the disintegration of chloroplasts in the cortical parenchyma cells of the petioles. The observations were subsequently confirmed, and bacteriological studies were started which resulted eventually in the cultivation from mosaic plants of organisms indistinguishable from those observed in the diseased chloroplasts. The seed transmission studies, which have a direct bearing on the bacteriological investigations, were started previous to the 1927 observations but have been amplified by the more recent work on the relation of the position of the seeds in the pod to infection with the mosaic virus. Although the investigational work is being continued, and much confirmatory work remains to be done before any degree of finality may be claimed for some of the findings, it is believed that the results that have been obtained, particularly in the bacteriological studies, if made available at this time, may encourage similar investigations with other virus diseases. The newer methods of investigation described by Hauduroy (1927) and Hadley (1931) need to be tested in their application to the implied possibility that the viruses are filterable forms of bacterial organisms. These methods, as yet unused

in virus plant disease studies, may be helpful in throwing some light on the obscurity which surrounds the causal agents of these diseases.

The first part of this paper deals with the general investigations of bean mosaic, part of which have a direct bearing upon the cytological and bacteriological studies that follow. The investigational work with rugose mosaic on the Refugee variety, as a matter of convenience and to avoid confusion, is discussed in a separate section.

TYPICAL BEAN MOSAIC

THE DISEASE

The term mosaic has become associated with the concept of a very characteristic type of disease in plants. The immediate connotation of the word is one that concerns contrasting shades of green and yellowish-green colors in the diseased leaves. In recent years, many diseases which bear little resemblance to the true mosaic troubles have been described and classified as maladies belonging to the mosaic group. A wide latitude in the use of the term has been the natural result of the discovery in rapid succession of a large number of diseases which possess some characteristics of the true mosaics but which do not necessarily belong to this group. Many of these diseases show undoubted relationship to the mosaic disease of tobacco as described by Mayer; in other cases, this relationship is not so clear. Until other methods are developed, the classification of virus diseases largely on the basis of symptoms will, of necessity, have to be continued.

Fajardo (1930) believes that there is only one mosaic disease affecting the bean. The writer has some evidence that a disease found so far only on the Stringless Refugee variety is distinct from the true mosaic to which this variety is extremely susceptible. This disease has been under observation for four seasons and the constancy of symptoms has prompted, tentatively at least, the consideration of it as a different type of mosaic. Additional work may show its relation to the common type of bean mosaic. For the sake of clarity the disease is described and discussed in a separate section of this paper. The greater part of the paper, however, deals with the classical or true type of mosaic disease.

HISTORY AND DISTRIBUTION

Bean mosaic was observed in Russia by Iwanowski at least as early as 1899, for he states in his discussion of the mosaic disease of tobacco (1899), "Ausser der Tabakspflanze habe ich die Mosaikkrankheit noch auf einer Sorte von Phaseolus vulgaris gefunden."

Clinton (1908) observed what he thought was an infectious chlorosis of beans on Connecticut. His photographic record of a diseased plant reveals an undoubted case of typical bean mosaic. The disease was reported by Stewart and Reddick (1917) as causing serious losses in New York state in 1916, and the general and severe epiphytotics in 1916 and 1917 probably prompted the investigations of these workers, whose first published observations appeared in 1917.

The first real contribution to our knowledge of bean mosaic was made by Reddick and Stewart (1918) in their report on varietal susceptibility. In this work, the infectious nature of the disease was demonstrated and a suc-

cessful method of artificial inoculation described. In a second report (1919) these workers amplified their previous list of varieties susceptible to the disease and a short time later (1919) submitted proof of the seed transmission of the disease as well as observations on the thermal death point of the embryo and the thermal resistance of the virus within the seed.

Stone and Howitt (1920) reported bean mosaic as a comparatively new or rare disease in Ontario in 1920. In the same year, Archibald (1921) observed as high as 25 per cent of the plants affected with the disease in some Canadian bean fields. Barss (1921) states that bean mosaic was first called to the attention of the station pathologist in 1917 and expressed the belief that it had been present in Oregon from an earlier date.

The origin and history of the Robust bean was described by Spragg and Down (1921) who state, "It originated from a healthy individual plant that was selected from among a lot of commercial beans containing mosaic in 1908." Since the disease was as yet undescribed in 1908, the statement that mosaic was prevalent in the planting from which the selection was made is based on retrospective assumption. It is not improbable, however, that mosaic was present in the field from which the selection was made and that the plant was taken because of certain desirable commercial characteristics which included, although unsuspected at the time, resistance to mosaic.

Mosaic was found affecting beans in Bermuda in 1923 by Ogilvie (1924); and, in 1926, Porter (1926) observed the disease in Eastern China. Pierce and Hungerford (1929) have presented some interesting evidence concerning the early occurrence of bean mosaic in the United States. In testing the viability of seeds in a collection of bean varieties made in 1897-1899, they planted one black wax variety, No. 24, that produced two plants in the green house test. One of these plants showed evidence of mosaic infection in the two simple leaves and the mottling became more intense as the compound leaves developed. They conclude that mosaic was present in this variety as early as 1899 and that the virus had survived in the seed for at least 30 years. Under ordinary storage conditions, bean seed rarely remains viable for longer than five years (Anonymous, 1920). In 1922, various lots of bean seed that had been stored in Mason jars for several years were tested for mosaic infection. These lots of seed were all of the Early Wonder variety and were from seven to nine years old. Of the nine year old seeds, only three germinated and one of the seedling plants showed clear evidence of mosaic infection. Grainger (1929) recently reported the appearance of bean mosaic in England. Although he thinks this is the first report of the disease in that country, the tests conducted at East Lansing in 1922 indicate that the disease has been present for a much longer time.

The mosaic disease of the bean is now world-wide in its distribution and is probably co-extensive with the host. The 1922 tests of disease resistance conducted at East Lansing by Rands and Brotherton (1925) with several hundred varieties of beans from all parts of the world afforded an opportunity to make some observations on the occurrence of mosaic in the seedlings. The observations were made at about the first to second compound leaf stage and before insect spread of the disease occurred. Symptoms of mosaic were observed in young seedlings of beans from the following countries: Argentina, Belgian Congo, Brazil, Chile, Colombia, Czechoslovakia, Ecuador, England, France, Germany, Guatemala, Holland, Honduras, Italy, Japan, Java, Africa, Mexico, Peru, Russia, Uruguay, and Venezuela. These observations indicate that the disease may be found wherever the bean plant is grown. In the United States, mosaic has been reported from 42 states. Since it is

an important trouble in all of the seed producing areas, it is to be expected that infected seed would carry the disease into all sections where beans are grown.

ECONOMIC ASPECTS

Before Robust, a mosaic-resistant navy pea bean was available for general planting in the eastern and mis-western bean States, mosaic was one of the most important diseases affecting the white bean crop. In 1916 and 1917, the disease caused severe losses in New York and also in Michigan. In 1919, it was reported to the Plant Disease Survey, Washington, (1920) from 25 states as causing reductions in yields ranging from a trace to 15 per cent. In New York, the estimated reduction in yield was 87,000 bushels. The adaptability of the resistant Robust bean to Michigan and New York conditions undoubtedly saved the white bean industry in these States. Since Michigan produces about 85 per cent of the entire crop of small white beans, the importance of this variety in making the continued production of pea beans profitable can hardly be over-estimated.

In some of the western States the Robust variety is not adapted to the climatic and cultural conditions of these regions and other kinds, like Great Northern, are more suitable. No resistant varieties have yet been produced for use in these States. In these districts, mosaic continues to be a very important disease. In the southern Idaho bean district, Harter (1927) found fields of beans in August and September showing as high as 85 per cent infection. Pierce and Hungerford (1929) state that the mosaic disease became such a decided menace to the bean industry in Idaho that in 1926 a bean certification program was undertaken under the direction of the State Seed Commissioner. In some of the other western States where the bean industry is an important one, mosaic is equally as serious as in Idaho.

The Stringless Refugee bean is the most popular and extensively grown variety of green snap bean for canning. Seed of this bean is largely produced in Colorado, Idaho, and other western States. It is very susceptible to mosaic and the disease is yearly an important factor in reducing the yields. Zaumeyer (1930) in 1929 noted from 10 to 100 per cent infection in this variety in the western seed-producing areas. Almost 100 per cent infection, accompanied by dwarfing and a marked reduction in yield, was observed in fields of Stringless Refugee in Michigan in 1929. Because Stringless Refugee plants produce a fair crop of pods when affected with mosaic, the reduction in yield due to the disease is frequently overlooked. By comparing healthy plants of this variety with others affected with mosaic, the serious effect of the disease upon yield can be observed. Apparently, the stock seed of this bean is now generally affected with mosaic and the disease seems to be on the increase. Tests conducted with this variety, and many others in 1922 indicated that the seed stocks were then fairly clean with respect to mosaic. Only one lot of Stringless Refugee seed was found to have as high as 17 per cent mosaic infection. Most of the samples were much freer of the disease. Tests made again in 1928 and 1929 revealed a very great increase in the per cent of seed infection since 1922. Some samples showed almost 50 per cent seed infection. Horsfall (1930) in July, 1930, examined 42 fields of string beans grown for canning in New York and found in 32 of them from a trace to 100 per cent of the plants affected with mosaic. He estimates at least a 10 per cent loss in the canning crop due to mosaic. This would be a very conservative estimate of the losses caused by the mosaic disease in the Michigan canning crop.

Most of the better varieties of snap beans grown for market purposes are susceptible to mosaic. In 1922, approximately 250 samples of beans were obtained from seven of the leading seedsmen who list beans as one of their specialties. Included in the pound samples of seed were all of the important bean varieties grown for market and for canning. All of these were planted on June 1 and the plants were carefully examined when the first compound leaves had fully expanded. From this test, it was evident that bean seed stocks in 1921 were singularly free from mosaic infection. One or two varieties were seriously affected with mosaic, but the great majority of the samples submitted showed less than 3 per cent infection and many were apparently entirely free from the disease. Considerable subsequent increase in the amount of mosaic infection has occurred in some of the varieties. Some of the popular market varieties are also grown for canning and in a number of fields observed in 1929, grown from seed produced in Colorado and other western States, the amount of mosaic was much greater than had been observed in the same varieties in 1922. In general, however, mosaic is much less important as a seed-borne disease in the market varieties of green and wax snap beans than in the canning sorts.

SYMPTOMS

It is impossible to state with any degree of certainty whether or not the wide range of mosaic symptoms that may be observed in any one variety or collection of bean varieties is due to one or more than one virus. While it has been suspected that there is more than one virus responsible for the diverse symptomatology that characterizes bean mosaic in its various stages, no one has yet proved by exhaustive inoculation tests that more than one virus is concerned in this complex of symptoms. Merkel (1929) has attempted to separate bean mosaic into three distinct types purely on the basis of symptoms. Although he has not carried out extensive inoculation studies, Fajardo (1930) has expressed the opinion that there is only one common type of mosaic of the bean. Because of the influence that variety, soil, temperature, and light exert upon the expression of symptoms, there is a great variation in the appearance of mosaic bean plants grown under diverse environmental conditions. Until more is known about the nature of the causal agents, the rapidly growing list of virus diseases is likely to be greatly augmented if there is a continuance of the tendency to separate these diseases on the basis of symptoms which, in many cases, are far from constant. A classification of these diseases on the basis of the reactions of the viruses as proposed by Johnson (1927) will probably separate more accurately the various kinds of disease on the specific hosts. Even a classification of this kind has its limitations, as shown by the changes produced in the virus of tobacco mosaic by high temperature (Johnson 1926) and the attenuation of the beet curly top virus by passage through a resistant host (Carsner 1921). Thus the reaction that a specific virus calls forth in its susceptible host may be fundamentally modified by various environmental factors and even by the host plant itself. The urgent need for concrete information concerning the nature of the viruses that cause these diseases is seen in the modifying influences that external and internal factors exert upon the type of symptom complex that is exhibited by a plant when it is invaded by a virus sensitive itself to these factors.

Although the classification of virus diseases on the basis of symptoms is a superficial and unsatisfactory one, no other has yet been devised to meet

the need of some simple way in which these diseases could accurately be separated. Some of the studies reported in this paper are based on the work with a disease that is apparently limited to the Stringless Refugee variety. It is similar in some ways to the true mosaic found on many other varieties of beans, but the foliage symptoms are unlike those of typical bean mosaic. Because the chief characteristic of the disease is a severe ruffling of the foliage, it has been designated as rugose mosaic and, for the present at least, classified as distinct from the true mosaic disease. Since the navy pea and Refugee beans are very susceptible to the true mosaic disease and show a great diversity of symptoms, mosaic will be described as it occurs on these varieties.

Mosaic symptoms may sometimes be detected in the simple leaves of bean plants grown from infected seed (Plate I, A). It is evident as a typical color mottling of light and dark green and is usually unaccompanied by any other modification in the normal appearance of the leaf, although some curling may be evident. Sometimes, only one of the simple leaves may exhibit the mottling. The simple leaves may show no mottling but exhibit a general chlorosis in comparison with the leaves of healthy plants. Clearing of the veins is in some cases the first noticeable symptom and may be observed in the simple leaves. Occasionally, the simple leaves are curled, are ruffled along the edges, or show some of the other symptoms that usually develop only in the compound leaves. The great majority of them, however, do not develop any symptoms of mosaic and have a normal appearance even on badly diseased plants.

The primary symptoms of mosaic rarely fail to appear in the first compound leaf in plants that are diseased from the seed. The earliest symptom to be observed is either a slight downward curling of the tips (Plate I, B) or a lengthening of the narrow apical portion of the leaflets. As development proceeds, the margins of the leaflets begin to curl downward also and typical mosaic mottling may appear in the laminae. The curling down of the marginal tissues of the lamina gives a decidedly arched appearance to the leaflet (Plate II, A). Under various inhibitory conditions, the mottling may be masked or confined to the apical portion of the leaflet and only the curling give evidence of the presence of the disease. The curling in some cases may be so severe that the opposite edges of the lamina are brought together on the dorsal side of the affected leaflet (Plate I, B2).

Another very characteristic symptom of mosaic on the first compound leaf and also the succeeding ones is the appearance of dark green blistered areas in the laminae (Plate II, A). This is especially noticeable on some plants in the field and is found more commonly on the navy pea beans. The blistering is practically always a contemporary symptom with the marginal curling of the leaflet. Both of these expressions of the disease are apparently due to localized disturbances of the synchronization of growth processes in the tissues of the leaflet, and are usually correlated with optimum growth conditions. When growth is retarded either or both of these symptoms may be lacking, but the curling is a more general symptom than the occurrence of the blisters.

One of the most interesting observations on the development of symptoms in the first compound leaf is the appearance of darker green areas along the veins with no mottling in the laminae. These dark green areas first appear at the extreme basal portion of the leaflet and gradually extend toward the apex, following the midrib and the lateral veins (Plate VI, B). The remaining portion of the leaflet is not chlorotic as might be suspected; the

dark areas along the veins are in many cases a deeper shade of green than the color of a normal leaflet. A slight downward curling of the leaflets may also be observed, but other symptoms are usually lacking. Chlorosis of the tissues adjacent to the midrib and lateral veins is not an uncommon symptom seen in the primary compound leaf (Plates I, B3; VI, A). The progressive development of either dark green or chlorotic narrow bands of tissue immediately adjacent to the veins would seem to be an indication of the movement of the virus through the vascular system. This conclusion is strengthened by other observations on the localized development of symptoms of the disease. Occasionally, plants are found on which the first compound leaf shows symptoms of mosaic in a part but not all of the tissues of the leaflets. One or two of the leaflets may be very noticeably mottled or dwarfed while the others develop normally (Plate VII, B). In Plate VII, A is shown a compound leaf with two of the leaflets very much dwarfed and the tissues adjacent to the lateral vein on the right hand side of the third leaflet showing the effects of the disease. Two-thirds of this leaflet, however, appears to be normal in every respect. The failure of some parts of the plant grown from diseased seed to show symptoms of mosaic is frequently observed and indicates a localization of disease processes in the plant.

The leaflets may be variously contorted without developing any mottling (Plate I, B4). In some cases, a slight waviness or roughness of the upper surface of the leaflets is the only detectable effect of the disease (Plate I, B 5).

As growth of a diseased plant continues and new compound leaves appear, the sequence of the symptoms that develop in the leaflets may show a great variation. In some cases, mottling may be the most conspicuous feature of the disease picture. Other plants may produce leaves in which the mottling is a very inconspicuous symptom, being overshadowed by the severe curling of the leaflets and a general dwarfing of the entire plant. During the hot weather of mid-summer, mottling seems to be masked to some extent and the leaflets tend to curl more. Mottling seems to be closely associated with rapid growth of the plant, and conditions which exert a retarding effect upon growth tend to suppress the expression of color contrasts in diseased leaves. Curling, however, seems to be accentuated by high temperature and for this reason this symptom appears to be dominant during the hot weeks of summer. Varietal reaction to the disease determines also to some extent the dominating type of symptom that is most characteristic of mosaic infection in the various varieties of beans. In the Stringless Refugee and some other very susceptible varieties, the chlorotic areas of diseased leaflets are frequently a pale yellow instead of a light green color. The islands of chlorotic tissues are very sharply demarcated from the darker areas which surround them, and are distributed irregularly throughout the laminae. In their placement they usually bear no relation to the veins.

In general, the leaflets are the only portions of a mosaic bean plant to exhibit the mottled and chlorotic effects of the disease. The petioles of a normal bean plant are a lighter green color than the leaflets. Sometimes mosaic bean plants are found in which the petioles show a faint mottling similar to that appearing in the leaflets. Unless a close examination is made this symptom escapes detection, since the color contrasts between the darker and lighter colored areas are much less pronounced than those in the leaflets. An explanation for this mottled effect in the petioles will be discussed in the section describing the cytological studies.

Aside from the stunting effect that mosaic has upon the growth of the

bean plant other systemic symptoms may also be observed. In plants diseased from seed, excessive branching is one of the commonly observed effects of mosaic (Plate II, A). Reduction in the length of the internodes is associated with the proliferation of branches, and the petioles are frequently shorter than those on normal plants. This stimulating effect of mosaic upon the vegetative buds has its counterpart in other diseases, like aster and peach yellows, where proliferation of a succession of vegetative buds produces a witches-broom effect on the affected plants. Associated with excessive branching is the delayed maturity that results from overstimulation of vegetative activity. Diseased plants are still green in the fall after mosaic-free plants have ceased vegetative activity and ripened their seeds. Thus we may have as the result of mosaic infection a general stunting effect upon the normal stature of the plant and a stimulating effect upon the processes which control the development and the proliferation of vegetative tissues.

One of the generally recognized effects of mosaic infection upon the reproductive activities of the bean plant is the failure of diseased plants to produce the normal number of blossoms. As a direct result of this partial sterility, fewer pods are formed on diseased plants. Shedding of the blossoms is another symptom of the disease under some conditions. Frequently, the young pods are shed after they attain a length of several millimeters. The shedding of the pods is due in all probability to the production of defective pollen, although other factors may also be concerned. In nearly all cases, fewer seeds are formed in the pods produced on diseased plants. In many cases only one very large seed is matured in a pod, and this is almost invariably located at the apical locus. One or more abortive ovules are present in the remaining loci. The sequence of seed formation in the pods is also irregular. Some pods will contain only seed number 1 others seeds 1 and 3, while still others mature varying numbers. The cause of abortion in some of the ovules has not yet been determined but is most likely due to defective pollen or egg cells. All of the enumerated results of mosaic infection have a direct effect upon the yield of the plant. The shedding of the blossoms, reduction in the number of pods, shedding of young pods, and the abortion of ovules are factors largely responsible for the decidedly smaller yield of seed obtained from diseased plants. Retarded growth and post-seasonal vegetative development result in many seeds failing to attain full maturity before frost, and this also influences the yield from mosaic plants.

Although mosaic infection generally has a suppressing effect upon flower formation, an anomalous condition has been observed in the navy pea variety where the disease caused a proliferation of the buds of the inflorescence (Plate III, A). This plant showed all of the typical foliage symptoms of mosaic and produced hundreds of flowers, all of which were sterile.

The symptoms that have been described are those that commonly develop in plants grown from infected seed. Plants that become infected during the growing season exhibit a great variety of symptoms, the time of infection, with respect to the stage of development of the plant, the seasonal environmental conditions, and varietal reaction determining the type and sequence of symptoms that appear. If infection occurs during mid-summer when the temperature is high, mottling is likely to be masked, but the cupping and curling of the leaflets will be a conspicuous symptom. If infection occurs early, mottling may be pronounced and the disease may run a course similar to that described in plants infected from the seed. If infection is delayed until near the time of flower production, mosiac usually has little or no effect upon the reproductive activities of the plant, although the vegetative

organs that are produced after infection occurs are dwarfed and show the systemic effects of the disease.

In some cases, the observational evidence indicates that plants infected late in the season may transmit the disease through the seed. Although it has been shown that the virus ordinarily will fail to reach the seed if infection occurs after the flowers are formed, this does not seem invariably to be the case. The Refugee varieties in rare instances appear to transmit the virus through the seed on plants that show no apparent symptoms of the disease up to the blossoming period. In general, the amount of disease transmitted through the seed is in direct proportion to the length of time that is required after planting for a given variety to mature seeds. That this is not an invariable rule is shown by the reaction of the navy pea varieties, mostly short-season beans, but which transmit the mosaic virus to a higher percentage of the seeds than many other varieties. The highly susceptible sorts, like the Refugee and navy pea varieties, transmit mosaic through the seed much more freely and regularly than the more resistant kinds of beans. This may be explained on the basis of the ability of the virus to multiply and move more freely in the tissues of the highly susceptible varieties, thus enhancing the chances of the virus reaching the ovule in time to infect the cells of the embryo.

The most typical symptoms of bean mosaic are noticeable in those plants grown under optimum light, temperature, and other conditions that stimulate rapid growth. All other conditions being equal, light appears to be the most important factor in influencing the expression of symptoms. During the winter months in Michigan, mosaic bean plants show only mild symptoms of the disease. From the middle of November to the middle of February, it is difficult to determine, in many cases, whether or not a plant is affected with mosaic. Temperature has little effect in intensifying the typical symptoms of the disease during this period. These observations are not in accord with those of Pierce and Hungerford (1929), who found that higher temperature greatly intensified bean mosaic symptoms in Idaho during the winter months. However, their tests were made in early November or late in March, and a similar effect is produced on mosaic plants in Michigan during a corresponding period. Because light conditions in Idaho are more favorable for growth during the winter months than they are in Michigan, it is easy to reconcile the discordant results obtained in these two States. Infected seed planted early in November will produce plants with fairly typical symptoms of mosaic in the first compound leaves. The leaves produced later, particularly in December, usually do not develop noticeable mottling and in many cases no curling is evident. Mosaic symptoms appearing on plants after the middle of February increase in intensity up to the time when the high temperatures of May and June cause some masking in the greenhouse-grown plants. In the field, under normal Michigan conditions, the most typical symptoms are seen in late June and early July. Typical symptoms may continue to appear in the growth produced during July and August, but high temperatures and drought conditions during these months frequently have a marked influence on the mottling and other characteristics of mosaic plants.

During July and August, plants of some varieties, free from mosiac infection, respond to abnormally high temperature by the development of symptoms resembling in some respects those characteristic of mosaic-infected plants (Plate II, B). No mottling is evident but the leaves develop a blistered and savoyed appearance that may be confused with mosaic. In

1921 and 1930, this condition was especially noticeable. To determine the effect of shading, tests were made by caging some of the affected plants. After the cages were placed over the plants, the leaves produced were normal and free from the savoyed appearance.

TRANSMISSION

By sap expressed from diseased plants

That bean mosaic was not highly contagious disease like tobacco mosaic was proved by the failure of Clinton to reproduce the disease in healthy plants by rubbing the leaflets with the juice from mosaic plants. The infectious nature of the disease was first demonstrated by Reddick and Stewart (1918) when they secured infection in healthy bean seedlings by rubbing and scarifying the dorsal surfaces of the young leaflets with pieces of crushed leaf tissue from mosaic plants. Subsequent investigations by others have shown that the disease is not easily transmitted in this way (Elmer 1925; Fernow 1925; Merkel 1929; Pierce and Hungerford 1929; Fajardo 1930).

The writer's experiments have given results which agree with those of these investigators in showing that bean mosaic is transmitted with difficulty and irregularly by the usual inoculation method of leaflet mutilation. In no case has more than a small percentage of infection been obtained by rubbing the surfaces of healthy leaflets with the sap from diseased plants. Pierce and Hungerford (1929) and Fajardo (1930) have worked out modified methods of leaflet inoculation which have given high percentages of infection in the inoculated plants. Both of these methods employ large quantities of crushed infected bean tissue as a source of the inoculum, and thorough maceration is secured by the use of quartz sand as an abrasive. Pierce and Hungerford applied quanities of this macerated material to the surfaces of the young leaflets and worked the sap into the tissues with a fine needle, while Fajardo rubbed the upper surface of the simple leaves with a mixture of macerated leaf tissue and quartz sand inclosed in cheesecloth. The disease was readily transmitted by both methods.

Success in transmitting the disease appears to depend, in part at least, upon the use of freshly expressed sap obtained from relatively large quantities of tissue. If the virus is generally distributed throughout all portions of the plant, the difficulties encountered in attempts to transmit the disease by the usual method of leaflet mutilation and the success that has been obtained by macerating larger quantities of tissue are not readily reconcilable. If, on the other hand, we assume that the virus is localized in certain portions of the plant, the difficulties and irregular results obtained in transmission experiments are more easily explained. That such a localization does occur seems probable from the evidence provided by the irregular manner in which the virus is transmitted through the seed and the development of localized symptoms in leaflets produced on plants grown from infected seed. This evidence will be considered in the seed transmission studies.

By insects

Evidence that insects were responsible for the field dissemination of bean mosaic was obtained in 1921. Mosaic-free seeds of 20 varieties of beans were planted in June, 1921, at a distance of approximattely five rods from another plot of several varieties showing severe symptoms of mosaic. The

spread of the disease from the mosaic plot to the beans in the disease-free planting was charted at weekly intervals throughout the growing season. The first diseased plant was found on July 15; one week later four more were affected and when the final observations were made on September 17, it was found that 19.5 per cent of the plants had become infected with the disease. Caged plants of susceptible varieties grown adjacent to mosaic plants in the disease plot remained free from the disease until frost killed them in September. This test indicated that insects were active in spreading the disease in the field.

Transmission of the bean mosaic virus was accomplished with the potato aphid, *Macrosiphum solanifolii* Ashm., in May, 1922 (1922). Some mosaic and healthy bean plants growing in the greenhouse near potato plants became infested with the potato aphid and, following colonization of the lice on these plants, mosaic spread very rapidly. The aphids were transferred from the mosaic plants to healthy bean plants grown in cages, and conclusive proof obtained that this insect is capable of transmitting the disease. Later similar results were obtained with caged, field-grown plants. The potato aphid colonizes freely on greenhouse-grown bean plants during the months of April and May. Attempts to colonize the insect during the summer months on plants in the field and on bean plants in the greenhouse during the autumn months have usually resulted in failure. If potatoes are grown in the same greenhouse with beans during the spring months, *Macrosiphum solanifolii* Ashm. will reproduce freely on the beans following a natural or an artificial infestation. This aphid has been used successfully in a number of transmission experiments. On April 29, 1925, 22 young bean plants of the Golden Wax variety were infested with this aphid which had been reared on mosaic plants. Ten aphids were placed on each plant. The plants were kept in cages and additional insects were placed on the young leaflets on May 6. Twenty insect-free plants of the same variety were left uncaged for checks. On May 20 the few remaining aphids were killed by spraying the plants with nicotine sulphate and the cages removed. No symptoms of mosaic were apparent on the plants which were in a vigorous growing condition. However, 5 days later 12 of the 22 plants had developed typical symptoms of mosaic, evident as a mottling and down-curling of the leaflets. None of the check plants became diseased.

Although the potato aphid is able to transmit the virus of bean mosaic to healthy plants, it apparently is not responsible for the rapid spread of the disease in the field. Under favorable conditions mosaic spreads very rapidly in the field, and there can be little doubt that insects are responsible for this dissemination, but the potato aphid has not been observed colonizing on field-grown plants. Despite careful search in seasons when mosaic was spreading rapidly in the field, aphids have not been found except in very small numbers. An occasional winged adult of *Aphis rumicis* L. has been collected but this insect appears to prefer other host plants. In the fall, it has been collected from nasturtium plants and large numbers placed on mosaic bean plants but in no case was colonization obtained. A similar experience followed attempts to use the peach aphid, *Myzus persicae* Salz., in transmission experiments. Fajardo (1930) has reported successful transmission of bean mosaic with both of these aphids. From personal observations, during seasons when mosaic has spread very rapidly in Michigan bean fields, no evidence has been obtained that any of the aphids tested are concerned in the wide-spread dissemination of the disease. During July and August of 1929 and of 1930, bean mosaic was observed to spread with much greater

rapidity than usual. A close search for aphids showed that these insects were almost, or entirely, absent from fields where the disease was being rapidly disseminated.

Only two kinds of insects have been observed in large numbers in Michigan bean fields during the years when mosaic has been severe. These insects are the bean leafhopper, *Empoasca fabae* LeB. and the red spider, *Tetranychus telarius*. The leafhopper was especially abundant during July and August of 1929 and 1930. During dry seasons leafhoppers and red spiders multiply very rapidly, and mosaic then is also more prevalent, according to my observations. Circumstantial evidence that either the leafhopper or the mite is responsible for the spread of mosaic is based on observations on the correlation between the rapid spread of the disease and the presence of large numbers of these insects on diseased plants. Dry seasons favor the multiplication of the leafhoppers and red spiders but are unfavorable for aphids. This fact combined with the observation of the rapid spread of mosaic during dry seasons has led to the conviction that some insect other than aphids is probably concerned in the dissemination of the bean mosaic virus.

During August, 1929, collections of leafhoppers from mosaic bean plants were made daily, and the insects were released in cages containing young bean seedlings. The insects were collected with the insect catcher described by Kunkel (1926). From 50 to 100 nymphs and adults were released in each of the cages containing the healthy plants. The leafhoppers did not colonize readily on the caged plants, and no transmission of the disease occurred. Although this test gave negative results, further attempts will be continued.[1] Pierce and Hungerford (1929) and Fajardo (1930) failed to secure transmission with this leafhopper.

By seed

Evidence of seed transmission of the mosaic disease in leguminous plants was first obtained by McClintock in the lima bean (1917). Reddick and Stewart (1919) proved that the virus causing mosaic in the common bush bean was transmitted through the seed. That the seed harvested from even the most severely diseased plants does not carry the virus with any mathematical regularity has been noted by a number of investigators. Working mostly with small numbers of seeds, Reddick and Stewart (1919) noted a great variability in the percentage of mosaic transmission in the seed from various plants. In one test where 100 seeds were used, 50 per cent of the seedlings developed the disease. Kendrick and Gardner (1924) report that approximately 13 per cent of the seed harvested from mosaic soy bean plants produced diseased seedlings.

Burkholder and Muller (1926) state, "Seeds from a bean plant affected with the true mosaic seldom give rise to 50 per cent diseased plants."

Merkel (1929) harvested and planted approximately 1,000 seeds from field-grown bean plants showing typical symptoms of mosaic. In the 800 seedlings that were produced from this seed, 34.5 per cent exhibited mosaic symptoms. Pierce and Hungerford (1929) studied the seed transmission of bean mosaic in plants that became diseased through seasonal infection and also in those that were infected throughout the season from diseased seed. Seeds from plants that became infected during the growing season transmitted mosaic to 33.07 per cent of the seedlings. Mosaic appeared

[1] In further experiments conducted in the summer of 1931 using large numbers of leafhoppers no transmission of mosaic was obtained.

in 48.6 per cent of the seedlings produced from the seed harvested from the plants that had been grown from infected seed. This last test was conducted with 1,205 seeds, of which 792 germinated. It is evident from these and other tests that about one-half of the seeds produced on mosaic plants that originate from diseased seed are not infected with the virus. No explanation has yet been offered to account for this peculiar and very irregular way in which the mosaic disease of the bean is transmitted through the seed.

The writer's experiments on seed transmission confirm in general those of other investigators. In 1924, seed was collected from a planting of navy pea beans in which all of the plants were affected with mosaic. Some of the plants had originated from infected seed while others had contracted the disease during the growing season. These seeds were not planted until June, 1927. From a planting of 1,000 of these seeds, 644 seedlings were produced, of which 116 developed symptoms of mosaic in the first compound leaf. Thus, approximately 18 per cent of the seeds were infected with the mosaic virus. It has been shown that the virus can probably survive as long as the seed remains viable, so that there was no reduction in the percentage of seed transmission of the virus through aging of the seed.

Great variability has been observed in the percentage of seed transmission of the bean mosaic virus in seed harvested from individual plants that have been diseased during the entire growing season. In plantings of seed harvested from a large number of mosaic plants, a fairly uniform rate of transmission of the disease has been observed. In one such test, seeds from a large number of Refugee bean plants affected with mosaic were collected in September, 1929. These seeds were planted early in June, 1930, and as soon as the first compound leaf had been formed all plants that did not show clear symptoms of mosaic were destroyed. All the plants left in this planting, therefore, had arisen from infected seed. In September, when the seed was mature, but before dehiscence of the pods occurred, several pods were taken from each of 50 plants and the seeds planted in the greenhouse. From the 632 planted, 507 seedlings were produced, of which 256 showed unmistakable symptoms of mosaic. A transmission of mosaic of 50.29 per cent occurred in the seed from these primary-infected plants. These results agree with those obtained in similar tests and check closely with those of Pierce and Hungerford (1929), who observed mosaic in 48.6 per cent of the seedlings grown from seed harvested from primary-infected plants of the Great Northern variety. The failure of half of the seeds harvested from primary-infected bean plants to transmit mosaic to the seedlings is not characteristic of only certain varieties but appears to be true of all susceptible sorts that have been tested.

No satisfactory explanation has been formulated to account for the irregularities of seed transmission in the mosaic diseases of leguminous plants. Perplexing seed transmission problems are also met with in mosaic diseases of plants in other families. No convincing proof of seed transmission in tomato and tobacco mosaic has been submitted and yet there is some evidence that the disease occasionally may be carried in tomato seed (Bewley and Corbett, 1930). One of the most interesting and anomalous conditions is seen in the transmission of cucumber mosaic through the seed of the wild cucumber, *Echinocystis lobata* T. and G., while the same disease is not carried in the seed of the cultivated cucumber (Doolittle, 1920). The transmission of the disease through the seed of the wild cucumber occurs in practically the same ratio as in the seed of the bean. The entire problem of

seed transmission of the virus diseases has not yet investigated except from the economic viewpoint. Duggar (1930) has made the suggestion that the virus of the true mosaic disease of tobacco is inactivated by adsorption to the seed storage proteins. In the experiments reported so far, complete inactivation did not occur when the ground up seeds were added to the virus extract. In bean mosaic, this hypothesis could not explain the failure of some seeds to transmit the disease while others from the same pod do produce mosaic seedlings. It would be difficult to conceive of two adjacent seeds in the same pod having qualitatively different chemical compositions.

Generally it has been assumed that the mosaic viruses are distributed throughout the entire plant, although this assumption is based almost entirely on the demonstration of the presence of the tobacco mosaic virus in the green and yellow areas of diseased leaves, the flowers, and other parts of the plant. Although a virus may be present in all parts of some plants, in others it may be more or less localized in certain tissues. This view has much evidence to support it. Localization of the virus of potato leaf-roll has been demonstrated by Blodgett and Fernow (1921); Kasai (1921); and Quanjer (1920). Blodgett and Fernow (1921) and Ducomet (1922) showed that different eyes from the same tuber would produce both healthy and leaf-roll plants. Gardner (1924) in a test with the Rural variety found that out of 183 hills producing mixed progenies, 63 per cent showed leaf-roll in the largest tubers and 58 per cent showed none in the smallest tubers. Bennett (1927), in ringing experiments with raspberry leaf-curl, demonstrated that the curl virus did not pass beyond the girdle if all of the phloem was removed. Storey (1928) has shown that the virus causing streak diseases of maize is present in the chlorotic areas of the leaves but not in the green portions.

Cook (1930) in his summary of studies on sugar cane mosaic states, "but the fact that we find variations in both thickness and cell structure in a single leaf and sometimes within a single area indicates that there may be an unequal distribution of the virus throughout an organ of the plant. This is substantiated by the many records of plants (especially tobacco plants) in which one side only showed the symptoms and of leaves in which the symptoms were more severe on one side than on the other."

Hoggan (1931), in mosaic transmission studies with *Myzus pseudosolani* Theobald, explained the inability of this insect to transmit tobacco mosaic on the basis of its failure to extract the virus from the tissues in which it feeds. She states: "It would appear, rather, that for some reason the virus is not available to the aphid in the tobacco plant; in other words that it may be a question of the distribution of the virus within the host."

A study of the feeding habits of the various insects that transmit mosaic diseases may show that there is a relation between the location of the virus in the plant and the ability of the insect to transmit the disease. This relationship may be so specific that only those insects feeding directly on the tissue where the virus is localized are able to serve as carriers.

Evidence that the virus causing the mosaic disease of the bean is also localized in the plant is seen in the irregularities of seed transmission and the failure of some leaflets or portions of a leaflet to develop symptoms of the disease. Especial attention has been given to the studies on seed transmission and the distribution of the virus to the various seeds in the pods produced on mosaic plants. In 1929, seeds were harvested from a large number of mosaic Refugee bean plants and were planted in June, 1930. When the first compound leaf was developed, all plants that did not show

Table 1.—Seed Transmission of Bean Mosaic. Greenhouse Index of Seeds from 50 Plants.

Plant	Pod	Seed	Mosaic
1	1	2†	+
		3	−
	2	1	−
		2	*
		3	−
	3	1	−
		3	−
		4	*
	4	1	*
		2	*
2	1	1	−
		2	+
		4	−
		5	−
	2	1	+
		2	+
		3	+
	3	1	−
		2	−
		3	−
		4	−
	4	1	+
		3	−
3	1	1	−
		2	−
		3	+
		4	−
	2	1	−
		2	−
		3	−
	3	1	+
		2	+
4	1	1	*
		2	*
		3	+
		4	+
	2	1	−
		2	−
	3	1	−
		3	*
		4	*
	4	1	−
		2	−
		3	*
		4	*
5	1	1	−
		2	*
		3	+
	2	1	+
		2	−
		3	+
		4	+
6	1	1	+
		2	*
		3	+
		4	+
		5	−
	2	1	−
		2	−
		3	−
7	1	1	+
		2	+
		3	−
	2	1	+
		2	*
		3	*
		4	−
		5	−
8	1	1	+
		2	+
		3	+
		4	+
		5	+
	2	1	−
		2	−
		3	−
	3	1	+
		2	*
		4	−
9	1	1	−
		2	−
		3	−
		4	+
		5	+
	2	1	−
		2	−
		3	+
		4	+
		5	+
	3	1	+
		4	−
10	1	1	+
		2	−
		3	+
		4	+
	2	1	−
		2	−
		3	−
	3	1	+
		2	+
		3	+
	4	1	*
		2	*
		3	*
		4	+
11	1	1	−
		2	+
		3	*
		4	*
	2	1	−
		2	−
		3	−
	3	1	−
		3	+
		4	+
	4	1	+
		2	*
		3	*
12	1	1	*
		2	*
		3	*
		4	*
		5	*
	2	1	*
		2	*
	3	1	*
		2	+
		3	−
	4	1	*
		3	*
		4	−
13	1	1	−
		2	−
		3	−
		4	−
		5	−
	2	1	−
		2	+
		3	−
		4	−
	3	1	−
		2	−
	4	1	−
14	1	1	+
		3	+
		4	*
	2	1	−
		2	+
		3	+
		4	*
		5	*
	3	1	+
		2	+
		3	*
		4	*
		5	*
15	1	1	+
		2	+
		3	+
		4	+
		5	+
		6	*
	2	1	+
		2	*
		3	+
		4	+
16	1	1	−
		2	+
		3	−
		4	+
	2	1	*
		2	*
		3	*
		4	*
		5	+
		6	*
	3	1	+
		2	+
		4	*
		5	+

*No germination.
†The seed in the apical locus of the pod is designated as number 1.

Table I.—Continued.

Plant	Pod	Seed	Mosaic
17	1	1	−
		2	−
		3	−
		4	−
		5	−
		6	−
	2	1	−
		2	+
		3	*
		4	*
		5	*
	3	1	*
		2	*
		3	*
		4	*
18	1	1	−
		2	+
		3	−
		5	−
	2	1	+
		2	+
	3	1	−
		3	+
		4	+
	4	1	*
		2	*
		3	*
19	1	1	*
		2	+
		3	+
		4	*
	2	1	−
		2	−
		3	*
		4	*
		5	+
	3	1	−
		3	+
		4	+
20	1	1	+
		2	+
		4	+
	2	1	*
		2	+
		4	−
21	1	1	+
		2	−
		3	+
		4	−
		5	*
	2	1	*
		2	*
		3	*
		4	*
	3	1	*
		2	*
		3	*
22	1	1	−
		2	+
		3	*
	2	1	*
		2	−
		4	−
	3	1	+
		3	−
		4	−
		5	+
	4	1	−
		2	−
		3	+
		4	−
		5	+
23	1	2	*
		3	−
		4	*
		5	*
	2	1	−
		2	−
		3	+
		4	−
		5	−
	3	1	+
		2	+
		3	*
	4	1	*
		2	*
		4	−
		5	+
24	1	2	−
		3	−
		4	+
	2	1	*
		2	*
		3	*
	3	1	+
		2	*
		4	*
		5	+
	4	2	−
		3	+
		4	+
25	1	1	−
		2	−
		3	+
		5	−
	2	1	*
		3	+
		2	+
	3	1	−
		3	*
		4	*
	4	1	*
		2	*
		5	*
26	1	1	+
		2	+
		3	+
	2	1	−
		2	−
		3	+
		4	+
	3	1	*
		3	*
		4	*
27	1	1	−
		3	−
		5	−
	2	1	−
		2	*
		4	*
		5	*
28	1	1	−
		2	+
	2	1	−
		2	+
		3	−
		3	−
		4	−
29	1	1	−
		3	−
		4	−
	2	1	+
		2	*
		3	*
		4	+
30	1	1	−
		2	*
		3	+
	2	1	*
		2	+
		3	+
	3	1	+
		2	*
		3	*
		4	*
	4	1	*
		2	+
		3	+
		4	*
31	1	1	−
		2	−
		3	*
		4	−
		6	+
	2	1	−
		3	+
		4	*
		5	−
	3	1	+
		2	+
		3	−
		4	+
		5	+

Table I.—Continued.

Plant	Pod	Seed	Mosaic	Plant	Pod	Seed	Mosaic	Plant	Pod	Seed	Mosaic
2	1	1	—	37...	1	1	—	42.	1	1	—
		2	—			2	—				—
		3	—			4	—				—
		4	—			6	—				—
	2	1	—		2	1	—		2		*
		3	—			3	—				—
		4	—			4	*				—
		5	—			5	*				—
	3	1	—						3		—
		3	—								—
		5	—	38...	1	1	*				—
		6	—			2	—				—
	4	1	—			4	—		4		—
		2	—			5	—				—
		5	—			6	—				—
		6	*		2	1	—				—
						2	—				—
3	1	1	*			3	—				
		2	*		3	1	—				
		3	—			2					
		4	—			3	—	43	1		—
	2	1	*		4	1	—				—
		2	—			2	—				—
		3	—			4	—		2		—
	3	1	*			5	—				—
		2	+								—
		3	—								—
	4	1	—	39...	1	1	+				—
		2	—			3	+				—
		3	—		2	1	+				—
		4	—			2	+				—
		5	—			3	+				—
	5	1	+			4	+		4		—
		2	—		3	1	—				—
						2	—				—
4	1	1	—			3	+				—
		2	—		4	1	—				—
		3	—			2	—				—
		4	—			3	+	44	1		—
	2	1	—		5	1	+				—
		2	—			3	+				—
		3	—						2		—
		4	—	40...	1	1	*				—
	3	3	—			2	—				—
		4	—			3	—		3		—
	4	1	*			4	—				—
		3	—		2	1	*				—
						2	+		4		—
5	1	2	—		3	2	+				—
		3	—			3	+				—
		4	—			4	—				—
		5	—		4	1	—		5		—
		6	—			2	*				—
	2	1	—			3	—				—
		2	—								—
		3	—	41 ..	1	1	+				—
		4	—			2	—	45	1		—
		5	—			3	+				—
	3	1	—			4	—				—
		2	—		2	1	—				—
		3	—			2	+				—
						3	+		2		—
						4	—				—
6	1	1	*		3	1	—				—
		2	—			2	—				—
		5	—			3	—				—
	2	3	—		4	1	—		3		—
		4	—			2	—				—
		1	—			3	—				—
	3	2	—			4	—		4		—
		1	—			5	—				—
	4	2	—		5	1	—				•
		1	—			2	—				—

Table 1.—Concluded.

Plant	Pod	Seed	Mosaic	Plant	Pod	Seed	Mosaic	Plant	Pod	Seed	Mosaic
46	1	1	−	48	1	1	+	50	1	1	+
		2	−			2	−			2	+
		3	−			3	−			3	+
		4	+			4	+			5	+
		5	+		2	1	+		2	1	+
		6	+			2	−			2	+
	2	1	+			3	+		3	1	+
		2	+			4	−			4	+
		3	+		3	1	−		4	1	+
		4	−			2	−			2	−
	3	2	*			4	*			3	+
		3	−		4	1	−			4	*
		4	+			2	*				
		5	−			3	*				
	4	1	−			4	−				
		2	−								
		3	−								
		4	−								
				49	1	1	+				
						2	−				
						3	−				
47	1	1	+			4	−				
		2	+			5	−				
	2	1	+			6	+				
		2	−		2	1	+				
		3	+			2	−				
		4	−			3	+				
	3	1	−			4	−				
		2	+		3	1	−				
	4	2	+			2	−				
		3	−			3	−				
		4	+			4	−				
	5	1	+			5	−				
		2	+								
		3	−								
	6	2	−								
		3	−								
		4	−								

clear symptoms of mosaic were removed, leaving only those plants that arose from infected seed. In September from two to six pods were taken from each of 50 plants and the seed from the various loci in each of the 177 pods carefully indexed in the greenhouse for mosaic. Each plant was examined for mosaic when the first compound leaf was completely developed and the young leaflets of the second were expanding. The number of the plant, pod number, the position of the seed in the pods, and the presence or absence of mosaic in the seedlings is given in Table I.

Summary of Table I

Number of seeds planted	634
Number of seeds germinating	509
Number of healthy seedlings	253
Mosaic seedlings	256
Per cent of mosaic	50.29

A study of Table I will show that there appears to be no uniformity in the distribution of the virus to the seeds in the various pods on the individual plants or to the seeds in each pod. Two pods taken from the same plant may receive the virus in entirely different proportions. All of the seeds in one pod may be infected with the mosaic virus while in the other pod only one seed may be infected or the pod may contain a varying number of diseased seed. Table II shows the distribution of the virus to the seeds

in the various loci. These results are taken from Table I and show the condition of the seedlings grown from the seeds in 175 pods that were harvested from the 50 plants used in the test.

Table II.—Distribution of the bean mosaic virus to seeds in the various loci in the pods. Data from 175 pods taken from 50 mosaic plants.

Seed Number	Mosaic	Healthy	Per cent Mosaic
1	66	72	48.1
2	59	56	51.3
3	59	53	52.6
4	42	43	49.4
5	25	23	52.0
6	3	6	33.33

The probability of a bean seed becoming infected with the mosaic virus appears to be independent of the position occupied by the seed in the pod. Very few pods on mosaic plants contain as many as six seeds, and the per cent of mosaic transmitted through seed number 6 might be greatly increased if a larger number of seeds had been used. Since only minor differences in the rate of transmission of mosaic are shown by seeds in all of the other positions, the decrease shown by seed number 6 is probably not significant. The great variation in the rate of transmission of mosaic through the several seeds in the same pod is shown in Table III. In this Table, the percentages of pods containing from none to five infected seeds are given.

Table III.—Mosaic transmission expressed as percentages of pods containing 0, 1, 2, 3, 4, and 5 infected seeds. Data from 167 pods harvested from 50 refugee plants, 1930.

Pod type	0 mosaic	1 mosaic seed	2 mosaic seeds	3 mosaic seeds	4 mosaic seeds	5 mosaic seeds
1—seeded	43.7	56.2				
2—seeded	21.3	38.2	40.4			
3—seeded	31.8	25.0	31.8	12.3		
4—seeded	8.5	17.1	34.3	14.3	25.7	
5—seeded	23.8	19.0	4.8	23.8	14.3	14.3

The average mosaic transmission for the entire population was 50.29 per cent. In the 1-seeded pods the population average was exceeded, but in all the other classes it was considerably less. These results furnish convincing evidence that the failure of a seed to become infected with the mosaic virus is independent of the position it occupies in the pod. It is difficult to reconcile the theory of the presence of the virus in all portions of the plant with the results of the seed transmission tests. Some other explanation must be sought which gives consideration to these facts. It does not seem likely that genetical factors are concerned in determining transmission of mosaic through the seeds in the various pod positions. Since the bean flower is largely self-pollinated, no explanation based on nuclear behavior is readily apparent, but the fact that mosaic is rarely transmitted through the seed, if infection occurs after flowering, argues for some influence that may have a genetical

origin. This phase of bean mosaic investigations is now being given attention and the results of these studies will perhaps throw some light on this interesting problem.

Vascular Anatomy and Seed Infection

The irregular way in which bean mosaic is transmitted through the seed and the development of localized symptoms of the disease furnish contradictory evidence to the idea that the virus is widely distributed in the plant and that it is present in all of the tissues. Since the seed transmission studies have given evidence that could not be reconciled with this theory, an attempt has been made to find another explanation which would be in harmony with the observed facts of seed transmission and of the development of localized symptoms. In the discussion of seed transmission of bean mosaic, a review has been given of evidence previously obtained by investigators in a number of virus diseases, which tends to show that some of the viruses are restricted to certain tissues of the host plant. In the section dealing with foliar symptoms of bean mosaic, it was pointed out that the virus appeared to be localized in certain portions of the plant. A tentative explanation of the pecularities of seed transmission and localization of symptoms may possibly be found in the assumption that the virus is restricted to certain tissues and that localization of the infection prevents or retards dissemination of the causal agent to all portions of the plant. It is assumed, in the beginning, that the virus moves through the vascular system and the weight of evidence indicates that this movement occurs through the phloem. With this assumption as a basis, a preliminary study has been made of the vascular anatomy of the petiole and pod, with the hope of finding an explanation for the irregular seed transmission of the disease. The anatomical studies are not complete enough to warrant final conclusions since the amount and location of anastomosing of the vascular elements has not been definitely determined.

The bean pod is a monocarpellary, unilocular, dehiscent fruit. When ripened, the normal ovary contains several seeds. In the very young flower, the carpel is a flattish structure which eventually becomes hollowed out and united along the margins to form a closed, semi-cylindrical organ bearing the ovules along the ventral suture. The ventral suture is formed by the union of the marginal tissues and corresponds to the margins of the primitive leaf from which the carpel is supposed to have been derived. The dorsal suture of the pod corresponds to the midrib of the primitive leaf. The inner tissues of the pod are analogous to the ventral surface and the outer tissues to the dorsal surface of the primitive leaf. The ovules are attached parietally by their respective funiculi to the ventral ovary wall. Starting from the apex of the pod, the successive ovules arise alternately from the opposite edges of the carpel, resulting in a bilateral arrangement of the seeds in the pod (Plate VI, E). This alternate attachment of the ovules to the opposite edges of the carpel suggests some interesting possibilities in the invasion of the embryo by the virus of bean mosaic and may, in part, explain the occurrence of infected and non-infected bean seeds within the same pod.

A preliminary study has been made by means of freehand sections of the vascular system in the petiole, leaf and pod. Plants with well developed, compound leaves were severed below the simple leaves with a sharp razor and immediately placed in a dilute solution of eosin. The dye is taken up rapidly and in a few hours permeates the entire tracheary network. The course of the bundles may then be followed by means of free-hand sections

made at different levels. In the petiole below the leaflets, there are nine large vascular bundles arranged in a seven plus two system. Seven of the bundles form the main vascular cylinder and one bundle is found in each tip of the crescent on the dorsal side of the petiole. These two bundles terminate in the stipules. The main vascular cylinder is reduced to three large bundles at the base of the leaflet. The central bundle constitutes the midrib while the other two form the main lateral marginal veins of the leaflet.

In studying the vascular anatomy of the pod, sections were made from pods of different ages. The fresh sections were mounted on glass slides and treated with concentrated HCl, followed by an aqueous solution of phloroglucin. So far as possible, the sections were made in a plane to traverse the funiculus, but due to the curvature which characterizes this organ, only occasional sections were obtained in which the vascular tissues could be followed from the ovary wall into the developing tissues of the ovule.

The placental tissues of the carpel are served by two separate vascular bundles (Plate VI, C). These two bundles are the analogues of the two main lateral veins of the leaf. Because of the alternate attachment of the ovules to these two bundles, half of the seeds have a different vascular supply from the other half. Thus in a pod containing four seeds, seeds one and three have a vascular connection to one bundle while seeds two and four are connected to the adjacent, parallel bundle (Plate VI, C, D). Although the anatomy of the pod has not yet been worked out in detail, the preliminary study seems to indicate that each seed may have an independent vascular supply due to the termination of definite vascular units in each ovule. The seeds have no direct vascular connection to the single large bundle that is located in the dorsal wall of the ovary (Plate VI, D). This bundle is the analogue of the midrib in the leaflet.

On the assumption that the virus of bean mosaic moves through the vascular tissues, the failure of some seeds to become infected with the virus can be explained by the type of arrangement that characterizes the vascular tissues of the pod. A study of Table I will show that the infected seeds occur in all positions in the pod and that the number of seeds transmitting the disease is extremely variable. All seeds in a single pod may be infected, while another pod from the same plant may contain only non-infected seeds. Between these extremes are found pods in which the number of infected seed is widely variable. If all of the sieve tissues of one of the ventral bundles in the pod were infected (assuming that the virus moves through the phloem) we would expect to find alternating seeds transmitting the disease. Many pods are found in which this appears to be true (Table I, Plants 16, 21, 42, 27, etc.). However, this is not true with other pods, and, in these cases, we must assume that the virus is present in only certain elements of the bundle, and that only those seeds having a direct connection with the infected elements will become infected. If further and more complete studies on the vascular anatomy of the bean plant confirm the hypothesis of an independent vascular supply for each seed, a satisfactory explanation could be formulated for the occurrence of infected and non-infected seed within the same pod. There is much evidence to support the view that the virus moves through the vascular system and it would not be beyond reason to assume that this movement in many cases may be restricted to certain bundles or even to isolated elements of the phloem in these bundles. It would be necessary to postulate the occurrence of different degrees of in-

fection, and the studies on the development of symptoms in the leaves furnish evidence that many plants diseased from seed show only localized effects of the disease. If we abandon ourselves to speculation, though not without some basis of fact, it may be suggested that an explanation for the diverse symptomatology of bean mosaic and perhaps other virus diseases is to be found in a localization of the virus in certain tissues and a wide variability in the quantity and distribution of the infected tissues in individual plants.

OTHER METHODS OF TRANSMISSION

Reddick and Stewart (1918) made the suggestion that pollen might carry the virus of bean mosaic to healthy plants. Merkel (1929) attempted to transmit the disease in this way but was unable to arrive at any conclusions due to the shedding of the blossoms after pollination. The bean flower being cleistogamous is an argument against the probability of infected pollen being a factor in the field spread of the disease. The possibility of pollen carrying the virus is being tested, and the results will be published at a later date.

In 1922, some mosaic bean plants were observed to be heavily infected with rust. A susceptible variety of bean was grown in the greenhouse and the young plants were inoculated with the urediniospores of the fungus, *Uromyces appendiculatus* (Pers.) Fries. Although rust developed abundantly on the inoculated plants, no symptoms of mosaic appeared in the leaves

VARIETAL, SPECIFIC, AND GENERIC SUSCEPTIBILITY

Reddick and Stewart first tested the common varieties of field and garden beans for resistance and susceptibility to mosaic and found considerable variation among the more commonly grown kinds. In general, few varieties showed resistance to the disease. Robust, a white pea bean, was found to be highly resistant or immune. Some varieties that appeared to be resistant were found to be susceptible in later tests. White Marrow, Michigan White Wax, and Prolific Black Wax were listed as resistant varieties. In field tests here in 1930, White Marrow and Prolific Black Wax showed severe symptoms of mosaic but Michigan White Wax gave indications of being resistant.

Rands and Brotherton (1925) tested a large number of bean varieties collected in all parts of the world for resistance to anthracnose and blight and recorded observations on the field reactions of these varieties to mosaic. These tests were conducted at East Lansing and the mosaic readings were made by the writer in connection with other mosaic studies conducted during the summer of 1922. The season was a favorable one for the dissemination of mosaic and the disease spread throughout the entire planting, consisting of more than 600 varieties. Some varieties escaped infection but all of the more valuable snap and pea beans, except Robust, were affected with the disease.

In the 1922 tests, several species of beans in the genus *Phaseolus* as well as some beans belonging to other genera were included. The seed was planted in a special mosaic plot in which the alternate rows consisted of pea beans grown from seed which had been harvested from mosaic plants. No artificial inoculations were made, reliance being placed on natural insect dissemination of the disease. As has been previously mentioned, the season was unusually favorable for the natural spread of mosaic. Readings were taken

late in the season after natural dissemination had ceased. The results are given in Table IV.

Table IV.—Generic and specific susceptibility to mosaic. Field test, 1922.

Common name	Scientific name	Susceptibility
Pole bean	Phaseolus vulgaris L.	+
Bush bean	P. vulgaris, var. humulis Alef.	+
White tepary	P. acutifolius Gray, var. latifolius Freem.	+
Adsuki bean	P. angularis Wight	+
Moth bean	P. aconitifolius Jacq.	+
Rice bean	P. calcaratus Roxb.	+
Urd bean	P. Mungo L.	+
Mung bean	P. aureus Roxb.	−
Sieva bean	P. lunatus L.	+
Lima bean	P. limensis Macf.	+
Scarlet Runner	P. coccineus L.	+
Hyacinth bean	Dolichos lablab L.	−
Horse bean	Vicia faba L.	+
Asparagus or Yard Long bean	Vigna sesquipedalis L.	+
Sword bean	Canavalia gladiata D. C.	−

One of the interesting and still unsettled questions about varietal susceptibility concerns the reaction of the Robust bean. Reddick and Stewart (1918), from their inoculation tests, believe this variety to be highly resistant, if not immune. A similar opinion has been expressed by other workers who have tested or observed the variety under field conditions. It seems certain that Robust, under ordinary field or greenhouse conditions, is refractory to infection, if the appearance of visible foliage symptoms is taken as an indication of the presence of the virus in the plant. Despite the failure to detect symptoms of mosaic in plants that have been inoculated artificially, there are some indications that this variety is not immune to the disease. During the summer of 1921, a ten-acre planting of Robust beans on the college farm was severely defoliated by bacterial blight. Rainy weather in late August and early September stimulated secondary vegetative growth and the leaves on many of the plants showed well-marked symptoms of mosaic. Typical mottling, dwarfing, and curling of the leaflets was observed. Not all of the plants were affected, diseased ones being intermingled with others that were normal in appearance. These observations indicated that Robust might be a masked carrier of the virus.

During the spring of 1930, some intervarietal grafting experiments were attempted, using Robust and Stringless Refugee beans. The Robust beans were obtained from the Farm Crops Department of Michigan State College and the seed was from a pure line. Using the method described by Leach (1929) Robust scions were grafted on mosaic Stringless Refugee plants after the compound leaves had appeared. A few unions were obtained but all of the grafts with healthy Refugee scions on Robust were unsuccessful. After union had occurred, and the new leaves appeared on the Robust tops, very definite mosaic symptoms appeared in the leaflets. Typical mottling and curling of the leaflets was evident and there was no doubt that mosaic had been transmitted from the stock to the scion. This grafting experiment has shown that the Robust bean is not an immune variety. Whether or not it is a masked carrier of the virus under field conditions must be determined by further grafting and inoculation experiments.

CYTOLOGICAL INVESTIGATIONS

The nature of the etiological agents associated with the various virus diseases of plants has been a problem to challenge the imagination of investigators since Iwanowski attemped to demonstrate the presence of visible organisms in the tissues of mosaic tobacco plants. To a large degree, the cytological method of attacking the problem has been followed by those interested in this question, but, as yet, no answer has been found to the question of causation. As a result of these investigations, however, some valuable contributions have been made to our knowledge of the cellular and tissue pathology of plants affected with mosaic disease. Definite cell inclusion bodies have been found associated with some of the mosaic diseases and a great deal of attention has been given to these structures. For a discussion of the literature up to 1928 and for a description of these cell inclusions in solanaceous plants, reference should be made to the work of Smith (1926) and Goldstein (1926).

In other than solanaceous plants, the so-called amoeboid or X-bodies, associated with some kinds of mosaic disease in tobacco and other related plants, have not been demonstrated, but in searching for them another type of cell inclusion has been found in the phloem of a considerable number of plants. Attention was directed to these inclusions when the writer (1922) reported their occurrence in bean and tomato plants affected with mosaic and in potato plants affected with leafroll. In general these inclusions consist of elongated, tapering or spiral, protozoan-like bodies and have then been found only in the sieve tissues of the plants in which they have been observed. The nature of these bodies, called "slime-bodies" by Strasburger, has not yet been determined and considerable confusion exists concerning their constant occurrence in some plants. There seems justification for the opinion, expressed by some investigators, that these structures have no connection with the presence of virus diseases in these plants, since they have been found in plants apparently free from all symptoms of disease. In any case, they do not seem to be analogous to the amoeboid bodies, and the weight of evidence is against the probability of their being either of diagnostic or etiological significance. They require further study, and the discovery by Johnson and others of the occurrence of masked carriers of virus diseases makes desirable further investigation of the relation of these inclusions to mosaic disease in solanaceous plants. In addition to the plants that have been enumerated, the phloem bodies have been found in anemone (Klebahn, 1926), sugar beet (Schaffnit and Weber, 1927), and in lettuce and dahlia (Brandenburg, 1929). In the sugar beet, they were not found in plants free from mosaic symptoms, neither were they demonstrated in apparently healthy *Vicia faba* L. plants (Schaffnit and Weber, 1927).

The cytology of tobacco mosaic has received more attention than any of the other virus infections, and most of these studies have been concerned with the occurrence and nature of the amoeboid bodies associated with this disease in all of its solanaceous hosts. Since similar inclusions do not occur in bean plants affected with mosaic, only a brief discussion of them will be attempted. By some investigators, these bodies have been regarded as a stage in the life history of the causal agent; by others they are classified as degeneration products of the diseased cell. The controversy is not settled but some recent observations by Smith (1930) and Sheffield and Smith (1930) on the formation of these bodies in tomato plants affected with

Aucuba mosaic, seems to indicate that they are not the etiological agent. They explain the formation of the amoeboid bodies, usually found close to the nucleus, as due to the aggregation of protein particles that are moving in the cytoplasmic strands. The cytoplasmic strands tend to anastomose in the vicinity of the nucleus, and to this fact is due the occurrence of the bodies near or around the nucleus. They have observed the fragmentation of the bodies, which give the reactions of proteins, and make the claim that they crystallize in old leaves. If this observation is substantiated, the possibility of the amoeboid bodies being a stage in the life history of some living organism would be very remote.

REVIEW AND DISCUSSION OF LITERATURE OF SPECIAL SIGNIFICANCE IN THE PRESENT INVESTIGATION

Dickson (1922) made a cytological study of mosaic in a number of plants, including the bean, and described a disintegration of the chloroplasts in the ground tissues as a characteristic feature of the disease. He observed that the destruction of the chloroplasts was accompanied by the appearance of very small hyaline bodies in rapid motion, motion more rapid than could be accounted for by protoplasmic streaming. A striking difference in the thickness of the laminae of the light and dark areas of the diseased leaves was also noted. Hypoplastic development characterized the light green areas, while in the dark green areas, the cells were either normal in size and number or hyperplasia had occurred and the cells were smaller but occupied a greater volume of space. The observations of Dickson on chloroplast disintegration agree with those of previous and subsequent investigators, and although Cook (1930) could find no evidence of chloroplast destruction in sugar cane mosaic, and believes it of doubtful occurrence in other plants except in old leaves, there is abundant evidence to show that this is a rather constant feature of mosaic disease in many plants.

Eckerson (1926) described flagellated organisms that she observed in the chloroplasts and phloem tissues of tomato, Hippeastrum, dahlia, and squash plants affected with mosaic. Within 24 hours after inoculating alternate, immature leaflets of tomato plants with filtered juice from mosaic plants, she was able to observe the appearance of very small flagellated organisms in the veins and adjacent mesophyll cells. The chloroplasts in the cells nearest to the veins showed early signs of dissolution, and within the lytic areas of the chloroplasts the organisms were in active motion. No mottling of the leaflets occurred previous to 10 days after inoculation, but during the interim between inoculation and the appearance of symptoms, progressive invasion and destruction of the chloroplasts occurred. What appeared to be spore forms of the organism appeared within 20 to 30 days after inoculation.

Some very interesting observations on changes occurring in the chloroplasts of mosaic tomato plants were reported by Sorokin (1927). She followed the degenerative changes by studies on sections of live tissue and showed that minute, hyaline inclusions in rapid motion could be demonstrated in the liquefying chloroplasts of the chlorotic areas in diseased leaves. The hyaline bodies were variable in size but did not exceed 2 microns in diameter and were surrounded by a definite hyaline halo. There was a progressive liquefaction of the chloroplasts, finally resulting in spherical, transparent vesicles inside of which were 5-18 of the hyaline bodies. In the trichomes of diseased leaflets were found enormous numbers of small granular bodies

that stained deep blue with gentian violet and eosin after fixation with Zenker's fluid. Sorokin compared the cell inclusions she observed in tomato with those described for some of the animal and insect virus diseases. She believes that tomato mosaic is caused by an organism, the properties of which are similar to those of the Chlamydozoa, or the Rickettsiae, and that the tomato inclusions possibly represent stages in the development of an organism of this kind.

Chemical analyses made by Brewer, Kendrick, and Gardner (1926) of mosaic tomato leaves showed that there was a marked reduction in the total carbohydrate content and that this reduction was mainly in the polysaccharides. Dickson (1922) had noted in his studies of the chloroplasts of mosaic tobacco plants that there was a deficiency of starch in the chloroplasts of the chlorotic areas. The results of the chemical studies and the cytological observations on chloroplast disintegration indicate that inhibitive or destructive processes are features that characterize localized tissue reactions to the mosaic disease. The lysogenic destruction of the chloroplasts in strictly localized areas is best explained by the presence of an organism capable of secreting a proteolytic enzyme. The fact that destruction of the chloroplasts occurs in restricted areas is an argument against the assumption of a general distribution of the causal agents in all the cells of the ground tissues. Taken as a whole, the facts that are known to the present time indicate that the mosaic viruses move mainly through the phloem but that it is in the ground tissues that the inhibitive and destructive effects of the disease are most manifest. That the chloroplasts in the assimilative tissues are the main locus of the inhibitive and destructive processes of the invading virus is indicated by both cytological and chemical investigations.

MATERIAL AND METHODS OF THE CYTOLOGICAL INVESTIGATIONS

All of the characteristic symptoms of the true mosaic disease of the bean are very clearly expressed on plants of the navy pea variety. This variety is very susceptible to the disease and symptoms are easily detected even in early stages of development. Most of the cytological work has been done with leaves and petioles from various strains of this bean. Some work has been done with plants of the Stringless Refugee variety. Plants were grown both in the greenhouse and in the field and the disease has been studied in all stages of its development.

The initial observations leading up to this study were made on sections of living tissue from the petioles of greenhouse-grown plants. The sections were cut in the longitudinal plane, mounted in water and studied with the oil immersion, apochromatic lenses. Plants showing typical mottling were selected for sectioning, and most of the work was done with freehand sections of living tissue, mounted directly in water or in the buffered solution described by Zirkle (1926). This solution is valuable for preserving the chloroplasts for several days. Sections were also mounted in a 1-10,000 Janus Green solution for the study of the chondriosomes, but satisfactory results were not always secured with this dye, even with the certified material. For differentiating small starch granules, the sections were treated with a solution of iodine in chloral hydrate.

A large amount of material was imbedded in paraffin after a preliminary examination of freehand sections. Some of the freehand sections were killed, fixed, and stained in stender dishes and treated in the same way as the

paraffin sections. The most commonly used killing and fixing solution was a chrom-acetic mixture to each ml. of which, at the time of use, was added one drop of a 2 per cent osmic acid solution. This solution is destructive to chondriosomes and its use obviated the necessity of differentiating these structures in the sections. The solution did not blacken the sections so that it was not necessary to use a bleaching agent in the subsequent treatment of the sections. After a thorough washing of the killed material, it was dehydrated as rapidly as possible and infiltrated in cedar oil. With bean petiole material the oil infiltration is superior to xylol but it has to be carried out at a temperature of about 40° C. Most of the sections were cut 5 to 10 microns in thickness. With mature petioles, the thinner sections are obtained with difficulty because of the refractory xylem elements which make the sectioning in the longitudinal plane very difficult.

Haidenhain's iron haematoxylin stain gave the most satisfactory results, but sections were also stained with Giemsa stain according to the method described by Wright and Skoric (1928). The staining reactions of diseased cells are so altered in some material that satisfactory preparations were not obtained by either method.

Cells and chloroplasts from healthy and diseased plants were examined in the dark field. This method proved useful in identifying certain types of inclusions not readily differentiated by other methods. The material was mounted in a special dark field cell and studied with a fluorite objective of N.A. 1.15 used in conjunction with a special condenser of N.A. 1.20. No funnel stops are necessary with this equipment and a greatly increased resolution is possible over that obtainable with equipment utilizing a smaller numerical aperture.

COMPARATIVE OBSERVATIONS ON SECTIONS FROM NORMAL AND MOSAIC PLANTS

If thin, tangential sections are cut at various levels through the cortical parenchyma tissues of petioles from mosaic bean plants, there will be found cells in some of the sections that have a decidedly chlorotic appearance. Only a few cells in localized areas may show this condition, much variation will be found in different plants, but in most cases it will be possible to find areas in which there is clear evidence of chlorosis. The chlorotic areas may be found as readily in plants showing only mild symptoms of mosaic as in those with indications of a more severe form of the disease. Extensive searching of many sections may be required to locate these areas in badly diseased plants, while they may be found with ease in others less severely affected. There appears to be no correlation between the intensity of the macroscopic symptoms and the occurrence of microscopic chlorotic areas in the petioles.

While examining tangential sections of petioles from mosaic pea bean plants in January, 1927, chlorotic areas were observed in which the chloroplasts were showing evidence of dissolution. Various stages of this process could be observed in the affected chloroplasts within a single parenchyma cell. Some of the chloroplasts were beginning to show small vacuolated areas, in others the lysis was more extensive, and almost complete dissolution had occurred in others. The lysis appeared to be progressive, and in the later stages the entire chloroplast seemed to collapse into a mass of amorphous, colorless, viscous material in which numerous, small, hyaline granules were imbedded. The granules were mostly diplococcoid in shape and appeared to be sur-

rounded by a distinct, colorless halo. In the vacuolated areas the granules were in rapid motion, but the motility was not always continuous. The granules appeared to exhibit definite, translatory movements although this has not been established with certainty. These chloroplast inclusions appeared to be living organisms, and bacteriological studies were begun with the objective is isolating and growing them outside of the plant.

The Normal Chloroplast and Its Inclusions

In a normal parenchyma cell from the cortex of stem or petiole, the chloroplasts are usually oriented around the borders of the cell with the flattened surface toward the cell wall (Plate VII, D). Considerable variation may occur, however, in the arrangement within the cell (Plate VIII, E). The normal chloroplasts are dark green in color and usually homogenous in appearance, except in the central portion where starch grains are located. The chloroplasts vary in size but usually range from four to six microns in diameter. Zirkle (1926) has studied the structure of the chloroplast and has found that a remarkable uniformity characterizes these organs in the higher plants. According to his interpretation, the ground substance of the chloroplast is in the form of a flattened, prolate spheroid which surrounds a large central vacuole. The granular appearance of the stroma is due to numerous pores which connect the surface with the central vacuole. The ground substances is protein in nature and the pigments are evenly distributed throughout the stroma.

The description given by Zirkle of the chloroplasts in the plants studied by him agrees in general with the writer's observations on the chloroplasts in bean plants. The granular appearance of the surface of the stroma does not appear to be due entirely to the pores connecting the surface and the central vacuole. With eccentric illumination, the hemispherical surface of the chloroplast appears to be papillate in structure, that is, slight protuberances are present that give the golf ball network appearance to the chloroplast when it is viewed from the curved surface. When the chloroplast is viewed from the side, the papillae are not visible.

In the leaflet, the palisade parenchyma cells are rectangular in shape and occupy approximately two-fifths the transverse diameter. The chloroplasts are closely appressed to the cell wall and are usually filled with starch grains. In most of the sections examined, no other inclusions have been found in the normal chloroplast.

In occasional plants, inclusions other than starch grains have been observed. In a previous paper (1922) some changes in the chloroplasts from the cortical parenchyma cells of mosaic plants were described. At that time, it appeared that the chloroplast inclusions, described as occurring in mosaic plants, were not present in chloroplasts from normal plants, and the writer is not yet certain that they occur in plants entirely free from disease. They were however, found in a few sections taken from plants showing no symptoms of mosaic and, therefore, these inclusions are described as those that may also be found in plants exhibiting no symptoms of the disease. They are found more commonly in plants affected with mosaic.

In the chloroplasts of cells from the cortical parenchyma of petiole and also from leaf parenchyma cells, elongated, flattened or cylindrical, tapering or spindle-shaped bodies may sometimes be observed (Plate VII, C). With favorable illumination, they may be detected in unstained sections but are most easily seen when strained with haematoxylin. They strain deeply, and

when treated with the iron-alum solution hold the stain with about the same tenacity as the nuclei. They are extremely variable in size, ranging from a fraction to three or more microns in length, with the average width less than one micron. Progressive stages in the development of these inclusions occasionally may be observed in the same section. The first evidence of their appearance in the chloroplast is the presence of deep-staining granules, in some chloroplasts only one, in others several to many. These granules appear to elongate, enlarge, and finally to fragment into the spindle-shaped bodies. Some of the adjacent cells contain normal-appearing chloroplasts and there is no synchronous development of these inclusions within all of the chloroplasts contained in a single cortical parenchyma cell. The chloroplast undergoes changes as the inclusions develop, finally disappearing as a distinct structure with the spindle-shaped bodies imbedded in the gummy, amorphous residue. The viscous mass of material containing the inclusions retains to some extent the original form of the chloroplast, with the exception that in many cases it is much larger, due apparently to the collapse of the gelatinous ground tissue and the spreading out of the residual material. The altered chloroplasts may be arranged around the borders of the cell or distributed throughout the lumen. The inclusions in many cases appear to have ruptured the surrounding membrane and to be free in the cell cytoplasm.

In mosaic bean plants, this type of chloroplast inclusion has been found much more frequently than in plants showing no symptoms of disease. They are not found in all mosaic plants and their occurrence appears to be coincident with the sieve tube inclusions. In many respects, they resemble the so-called slime bodies. They are much smaller, however, and are not flagellate in appearance. They are much larger and in other respects unlike the plastid primordia that have been found in other plants (Randolph, 1922). Apparently, they are not analogous to those structures. The fact that they occur much more frequently in mosaic plants suggests that their formation is determined by some of the metabolic disturbances associated with the disease. It is not inconceivable that similar disturbances may be initiated by other causes and that this accounts for the finding of these inclusions in occasional plants free from visible mosaic symptoms. Again, the apparently normal plants in which they have been found may be in the incipient stages of mosaic and it appears from the work of Eckerson (1926) on tomato mosaic that the chloroplasts are the first organs to show the effects of the invading virus. Our methods of diagnosis are, at present, too crude to insure a detection of mosaic infection until macroscopic symptoms appear.

Chloroplast Changes in Mosaic Plants

In addition to the inclusions that have just been described in the chloroplast from normal-appearing plants and the changes associated with their presence in these organs, some other inclusions and degenerative phenomena peculiar alone to mosaic plants have been observed. Chloroplast degeneration seems to be one of the microscopic symptoms of mosaic disease in many plants. The only contrary evidence presented so far is that of Cook (1930) who did not find any evidence of chloroplast destruction in sugar cane mosaic. He believes the chlorotic areas in the leaves represent hypoplastic areas where chlorophyll has failed to develop. The observations made in this investigation, in the bean, are in accord with those of Cook only in so far as they relate to the youngest leaves. Hypoplastic areas are found in

both the young and older bean leaves from mosaic plants but disintegration usually does not occur until maturation of the tissues has occurred. The disintegration is not by any means limited, as inferred by Cook, to tissues in which senescence has begun. Progressive stages in the development of chlorotic areas have been observed in both petioles and leaflets in which there was no evidence of senescence. In some cases, no disintegration of the chloroplasts is associated with the chlorosis but in others destruction of the chloroplasts is one of the contributing causes to the development and extension of the chlorotic areas.

Tangential sections through the petioles of mosaic bean plants are frequently very suitable for demonstrating destructive changes in the chloroplasts. In such sections, small islands of chlorotic cells, surrounded by normal-appearing cells, are to be observed in petioles from a majority of mosaic plants. Some of the islands are made up of only a very few cells, while others are more extensive. In the chlorotic areas, the chloroplasts may be fewer in number and smaller than those in the green portions, but, in some cases, there is no deficiency in number and the chloroplasts are normal in size. The affected cells are pale yellow in color and structural changes in the plastids are frequently to be observed. The stroma becomes flattened and larger in diameter and, as a result of dissolution processes, the chloroplast eventually collapses into a coherent mass of viscous, pale yellow or colorless material with a more highly diffractive surface membrane at the interface (Plate VII, D). Remnants of starch grains are sometimes present and vacuoles may also be evident. No other inclusions have been observed in this type of chloroplast destruction. Similar changes may be observed in the chloroplasts of the leaf parenchyma.

The first evidence of another type of chloroplast destruction is the appearance of one or more small vacuolated areas in the stroma. These vacuolated areas appear to originate at the surface of the chloroplast rather than from the interior. Within the vacuolated areas, small, hyaline granules are always present, at rest or showing continuous or discontinuous motion. In the earliest stages, a single granule surrounded by a hyaline halo appears to be imbedded in a portion of the gelatinous stroma from which the color has disappeared (Plate VIII, C). The imbedded hyaline body is spherical or diplococcoid in shape and resembles the chloroplast inclusion bodies described by Sorokin (1927) as occurring in the disintegrating chloroplasts of tomato mosaic.

Chloroplasts showing different degrees of lysis associated with the presence of the hyaline bodies may be observed in the same cell. The hyaline diplococcoid bodies increase in number as the stroma undergoes progressive dissolution. They appear to be in active motion, and this motility does not seem to be entirely Brownian in nature. If the sections are kept in water in the ice box for 24 hours or longer, the motility of the bodies appears to be lost; when again examined, they no longer give evidence of independent motility such as may be observed in the fresh sections. It is believed that the bodies observed in the chloroplasts are bacteria, and, since they have been repeatedly isolated from a large number of plants and grown in culture and in no cases have shown evidence of motility outside of the plant, the observational evidence which tends to show that they are motile in the chloroplasts cannot be considered as adequate. The diplococcoid bodies may exhibit a rapid and translatory motion with intermittent periods of immobility. This would seem to indicate that independent motility does occur but a

similar behavior is shown by granules that are present in the cell cytolplasm. The weight of evidence is against the probability that these bodies possess the power of independent motility.

The entire chloroplast may be destroyed by the disintegrative processes that are associated with the presence of the coccoid bodies. When this occurs, the cocci escape into the cell lumen and are present in large numbers within cells in some sections of the petiole (Plate VIII, B). In some cases, however, the disintegrative processes do not appear to alter completely the gelatinous nature of the stroma and the cocci remain imbedded in the residual mass of the chloroplast (Plate VIII, A). In the cholorotic areas of the leaflets, similar changes have been observed in the chloroplasts in both the palisade and spongy parenchyma cells. Disintegrative changes in the chloroplasts of the dark green areas have not been observed. The frequency with which the coccoid bodies may be found in the chloroplasts of the leaf parenchyma cells is approximately the same as that in the petioles. In some sections, they will not be found at all, even though the chloroplasts are undergoing disintegration. They appear to occur more frequently in the cells adjacent to the vascular strands and there may be considerable significance in this fact, for, in the bacteriological studies, it will be shown that these cocci are found in the seed and in all probability reach there through vascular invasion.

In sections from some of the plants that have been studied, extremely small, coccoid bodies, (less than 0.5 microns in diameter) in enormous numbers, have been observed in the chloroplasts of the palisade and mesophyll parenchyma cells of leaflets from mosaic plants. They can be observed only with the highest powers of the microscope and careful attention to the details of lighting with the substage condenser is necessary if they are to be seen at all. An oil immersion lens of high aperture should be used, and oil must replace the air between the upper lens of the condenser and the lower surface of the slide. Otherwise, they will not be resolved and made visible. It will be necessary also partly to close the iris in the condenser diaphram. Under the best conditions for maximum resolution, these tiny bodies can be seen individually as spherical, hyaline structures distributed throughout the entire chloroplast. This has been determined by focusing first on the upper surface and then downward through the entire chloroplast. The bodies are found in great numbers through the entire stroma of the plastid. They have not been demonstrated with any greater degree of regularity than the large coccoid bodies. None of the methods of killing, fixation, and staining has been successful in making these inclusions visible in the paraffin sections. This possibly may be due to their extremely small size and a staining reaction that can not be differentiated from that of the stroma of the chloroplast, or they may be destroyed by the killing and fixing processes. They have been found only in mosaic plants and have been observed in leaf sections from plants affected with both the true and the rugose mosaic diseases.

The nature of the larger coccoid bodies in the chloroplasts remained undetermined until 1928 when bacteria identical in morphology with them were isolated from leaflets of mosaic plants grown under sterile conditions. This alone does not prove that the chloroplast inclusions are bacteria, but the evidence strongly suggests that they are. The staining reactions are identical with those of bacteria, and the bodies have been found only in the chloroplasts of mosaic plants. They do not occur with any degree of regularity in the chloroplasts from mosaic plants and disintegration of the plastids may take place without any evidence that they are present. However, they have

been found only in chloroplasts that were undergoing dissolution and it is assumed that they were, in part at least, responsible for the destructive processes. From the evidence obtained in the bacteriological studies, the best explanation of the occurrence of these cocci in the chloroplasts is that they are present in the vascular system of mosaic plants and are able, under certain conditions, to invade the parenchyma tissues in the regions of the vascular strands and attack the chloroplasts in the cells of these tissues. From the observations on the initial stages of the dissolution of the chloroplasts, it seems that they are able to invade chloroplasts that have not yet shown evidence of disintegrative processes. For the present, we must be content with the knowledge that coccus-like organisms, under conditions as yet undetermined, can be demonstrated in the disintegrating chloroplasts of mosaic plants, but that dissolution of the chloroplasts also may occur without visible microscopic evidence that organisms are present.

The Coccoid Bodies in Other Tissues

Coccoid bodies, similar in all respects to those observed in the chloroplasts of the leaf and petiole parenchyma cells, have been observed in the parenchyma cells of the vascular strands. They have been found without difficulty in some sections; in others, they have not been demonstrated at all. The border parenchyma cells of xylem and phloem sometimes contain them in large numbers, both in the young and mature tissues (Plate IX, A-B). In some cases, they have also been observed in the sieve tubes. Frequently, the border parenchyma cells of a single vascular bundle are occupied by these bodies while they are absent from similar cells in other bundles. Even more marked localization has been noted in some sections where the cocci occur in the parenchyma cells for only a short vertical distance along the petiole. They occur singly and in pairs, as they have been observed in the chloroplasts and show the same variations in size.

The irregular occurrence and distribution of these coccoid bodies in the border cells of the phloem and xylem parenchyma is exactly what might be expected if they represent the cocci that have repeatedly been isolated from young seeds from mosaic plants. It was shown in the seed transmission studies that the virus itself is found in only a portion of the seeds from mosaic plants and that the irregular distribution of the virus to the seeds indicates a localization of the causal agent in the plant. The peculiarities of vascular anatomy have been pointed out as providing the best explanation for the failure of all seeds from primary-infected bean plants to receive the causal agent. In the bacteriological studies, it will be shown that the cocci are present in the young seeds from diseased plants in approximately the same percentage of cases that the virus is transmitted to the seeds. These facts indicate that the cocci, like the virus, must have a vascular portal of entry to the ovules and that the occurrence of coccoid bodies in the vascular tissues is consistent with the results that have been obtained in the bacteriological studies.

The chrom-acetic mixture plus a small amount of osmic acid was used as the killing and fixing solution because of the well recognized fact that it is destructive to mitochondria. Mitochondria occur generally in the cells of formative tissues, and the writer's observations indicate that in the bean these structures may be confused with the cocci, which apparently are entirely distinct bodies. In sections from healthy plants, fixed and stained in the same way, the coccoid bodies have not been seen in the tissues in which

they have been demonstrated in mosaic plants. The localization of the coccoid bodies in certain strands and their absence from others of equal development in the same section is evidence that these structures are unlike the mitochondria.

BACTERIOLOGICAL INVESTIGATIONS

REVIEW AND DISCUSSION OF LITERATURE

Iwanowski (1903) believed that very small bacteria were present in the leaf cells of tobacco plants affected with mosaic, and he attempted to isolate and grow them in culture. Since he considered the sap from the leaves of tobacco plants an unfavorable medium for the growth of bacteria, he used a synthetic solution incorporated in agar. He was successful in isolating from raw, unfiltered sap, expressed from diseased leaves, bacteria which he believed produced infection when inoculated into healthy plants. Only a small percentage of the inoculated plants developed the disease, and, in some of his experiments, all of the inoculated plants failed to show symptoms of mosaic. In the light of our present knowledge concerning the contagiousness of the disease and the chances for the occurrence of accidental infections in handling the plants, it seems improbable that Iwanowski was dealing with the causal agent.

Smith and Boncquet (1915) found a bacterial organism constantly associated with sugar beet plants affected with curly top. The organism was found in great numbers in the sieve tubes, but they were also able to isolate it from the surface of normal plants, from sugar beet seed, and from the soil. Its presence in the plant seemed always associated with foliage abnormalities and the plants unaffected with curly top from which it was isolated showed crinkling of the leaves or other morphological irregularities. They were unable to produce infection with the organism when they inoculated healthy beets with pure cultures.

Boncquet (1916) reported the presence of an exceedingly small *Streptococcus* in tobacco plants affected with mosaic. He also claimed to have found a similar organism in mosaic potato plants. He states that the organisms were active nitrate reducers and that the juice of diseased plants contained nitrites and ammonia. No description was given of the methods used in demonstrating these organisms in the mosaic plants so that no conclusion can be drawn from the mere statement that these organisms were present in the diseased plants.

In the stem tissues of potato and tomato plants affected with mosaic, Melhus (1922) reported the occurrence of certain bacteria and other organisms not found in healthy stem tissue. He states "The presence of organisms in mosaic-infected tissue is often markedly constant and probably largely responsible for the marked dwarfing." No amplified report of these investigations has been published and no conclusions can be drawn from the brief statement contained in the abstract.

Dickson (1922) observed what he believed to be bacteria in the border parenchyma cells of the vascular tissues of the leaves of mosaic tobacco plants. The organisms were approximiately 0.3 microns in diameter and were present in the affected cells in enormous numbers. Dickson considered them identical with the bodies described by Iwanowski and possibly similar to the small *Streptococci* reported by Boncquet in mosaic potato and tobacco

Plate I

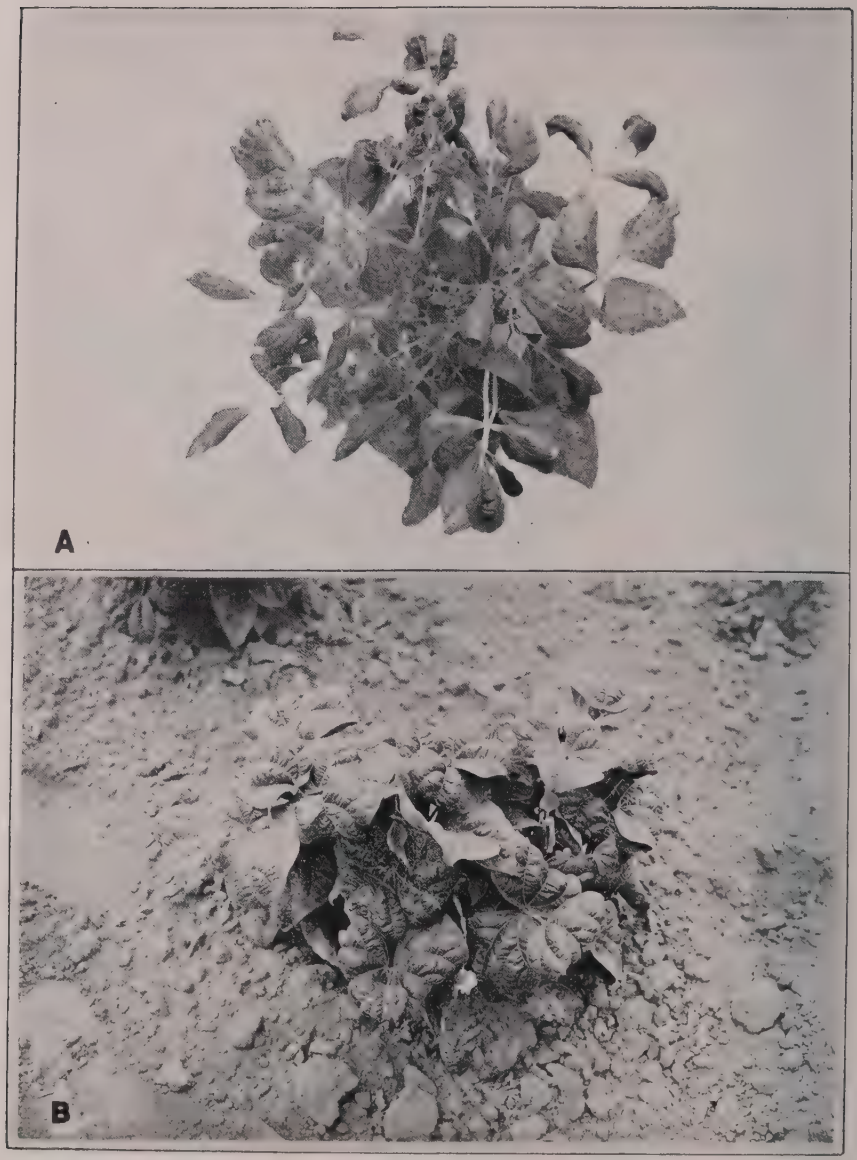


Plate II

Plate III

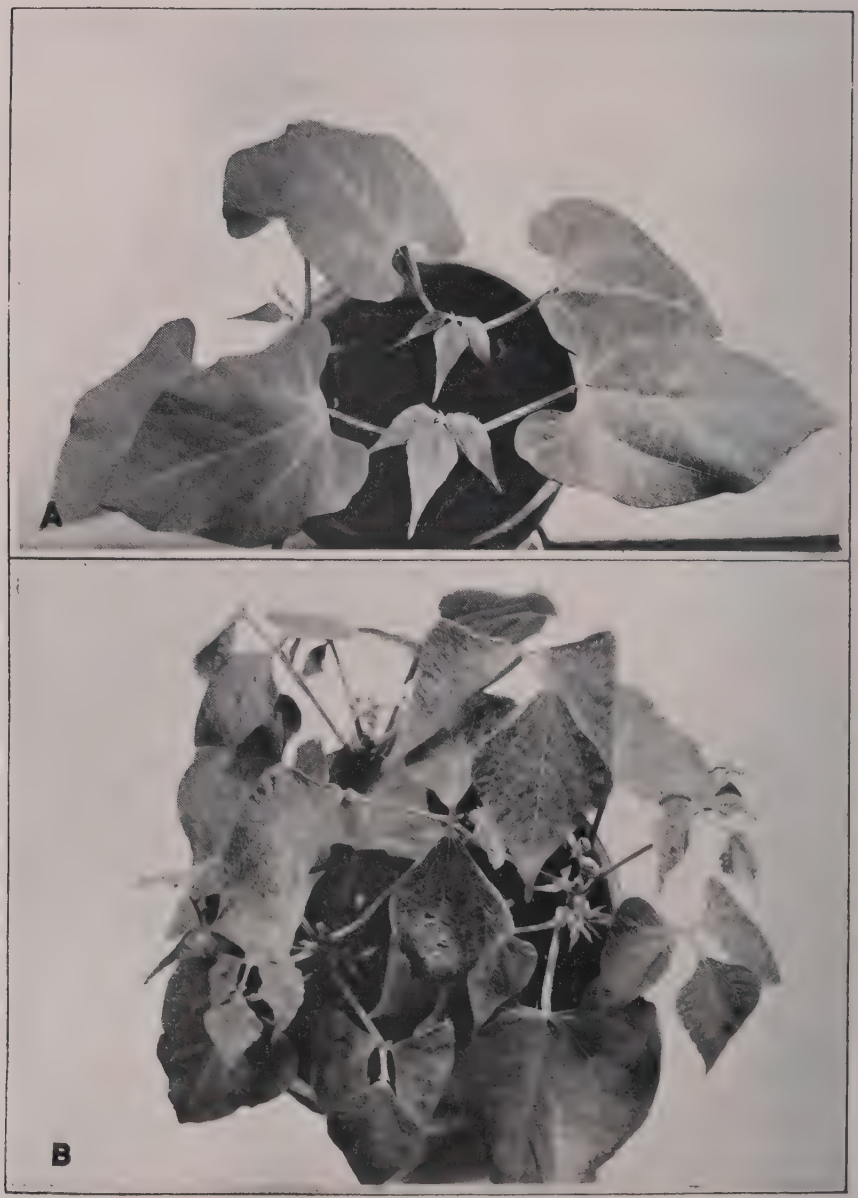


Plate IV

Plate V

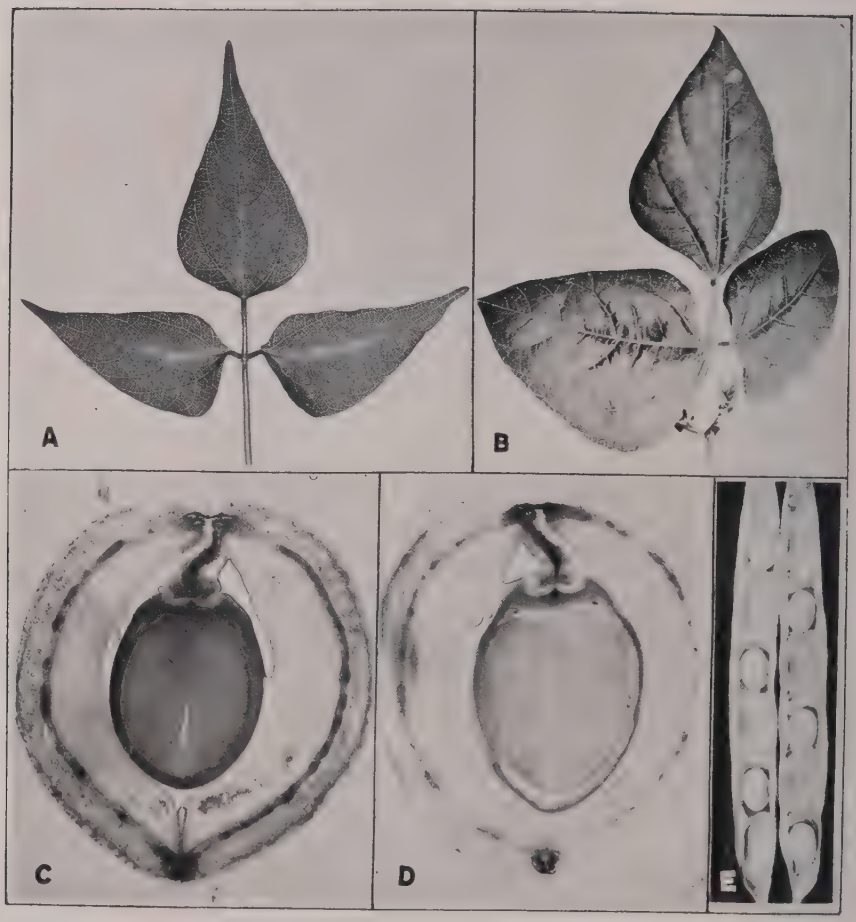


Plate VI

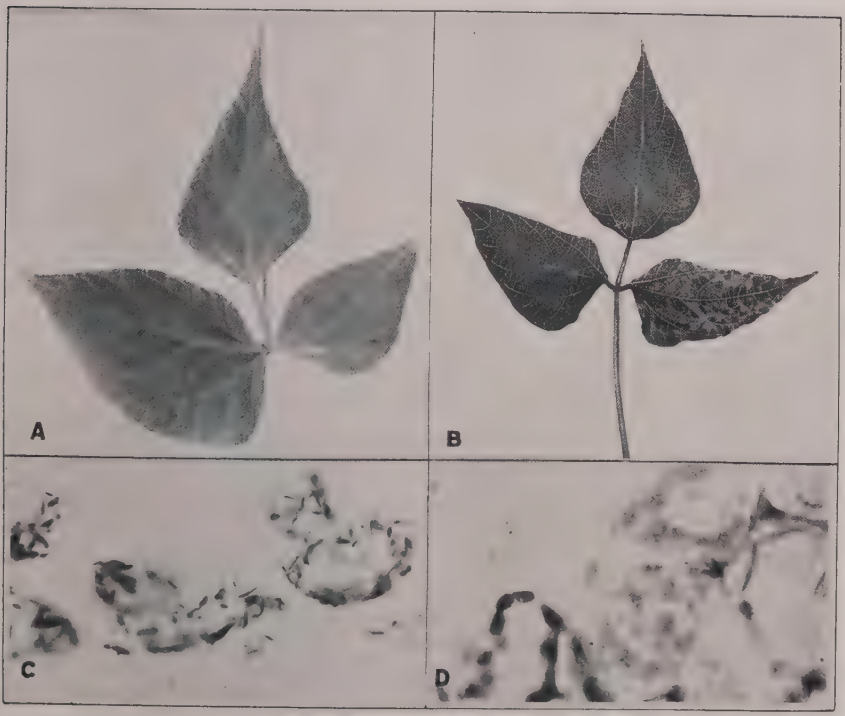


Plate VII

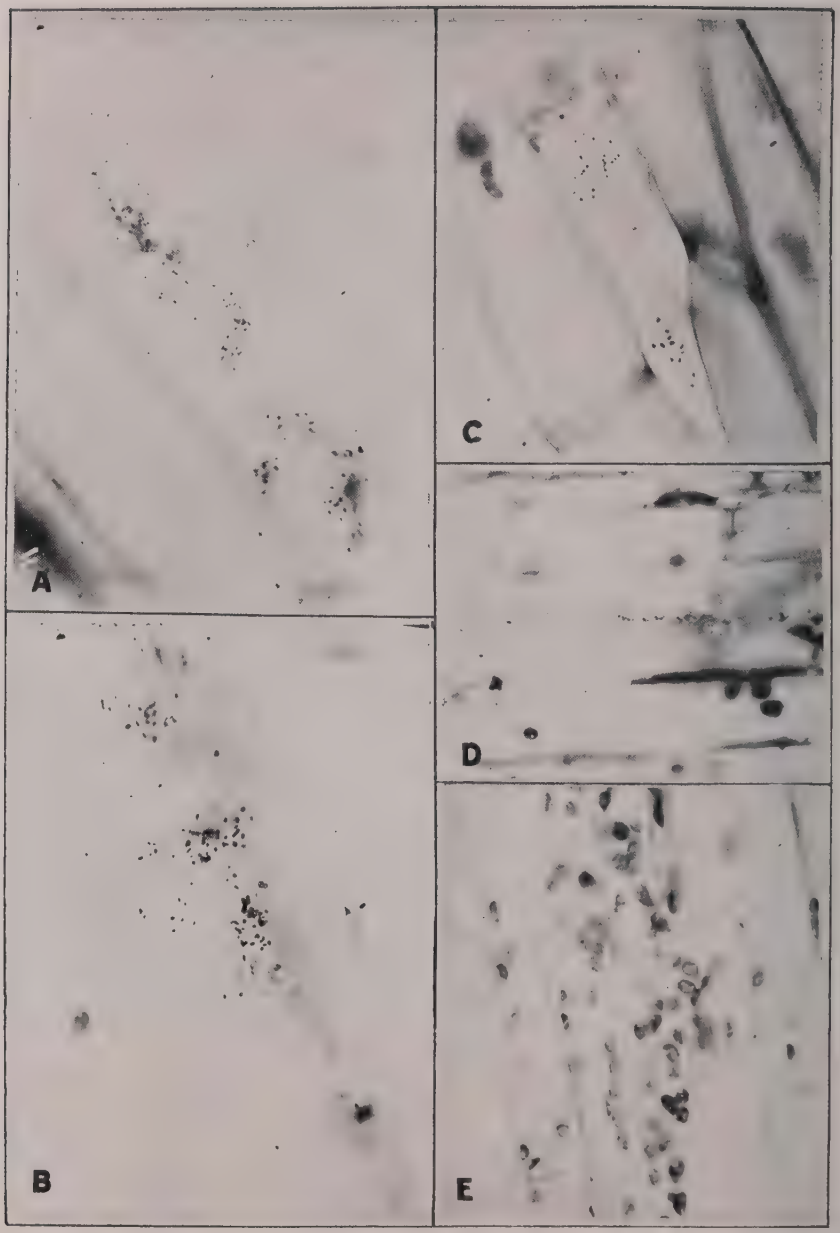


Plate VIII

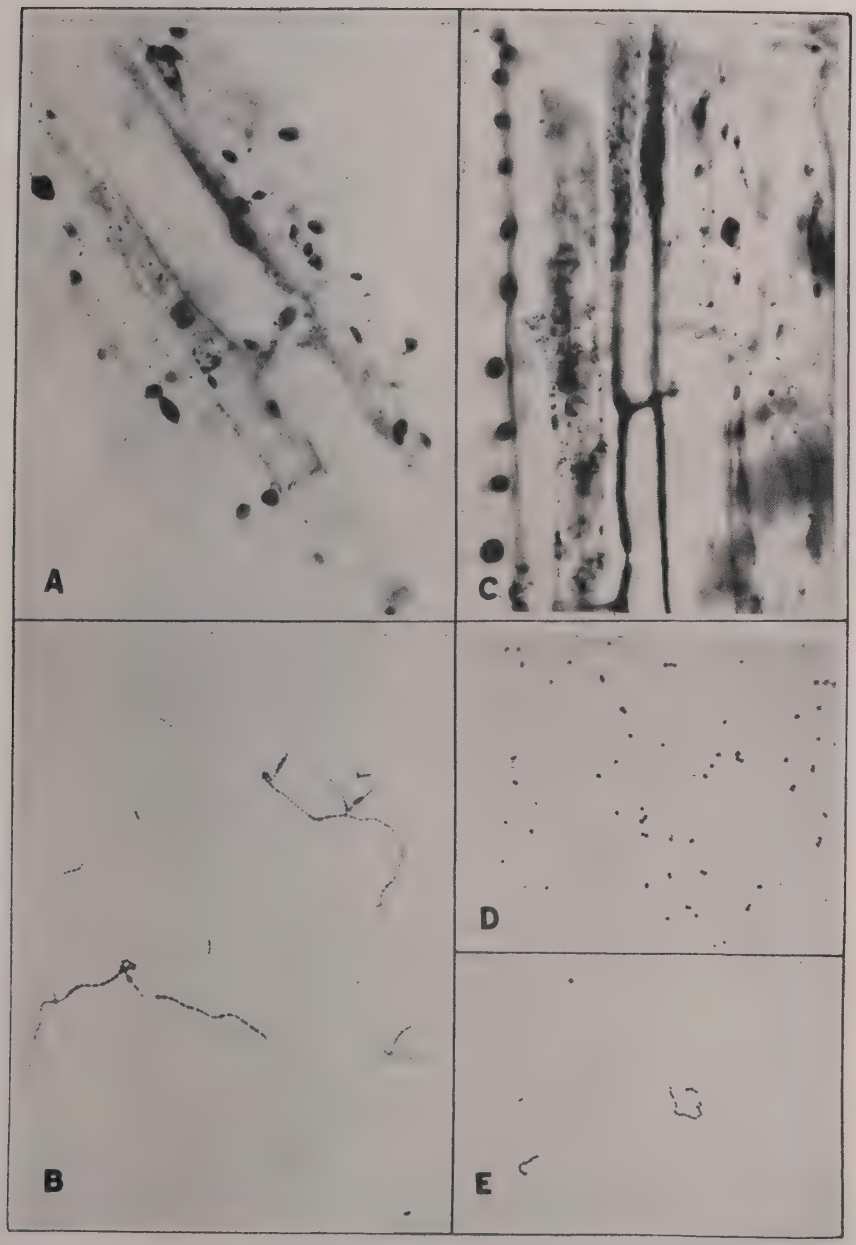


Plate IX

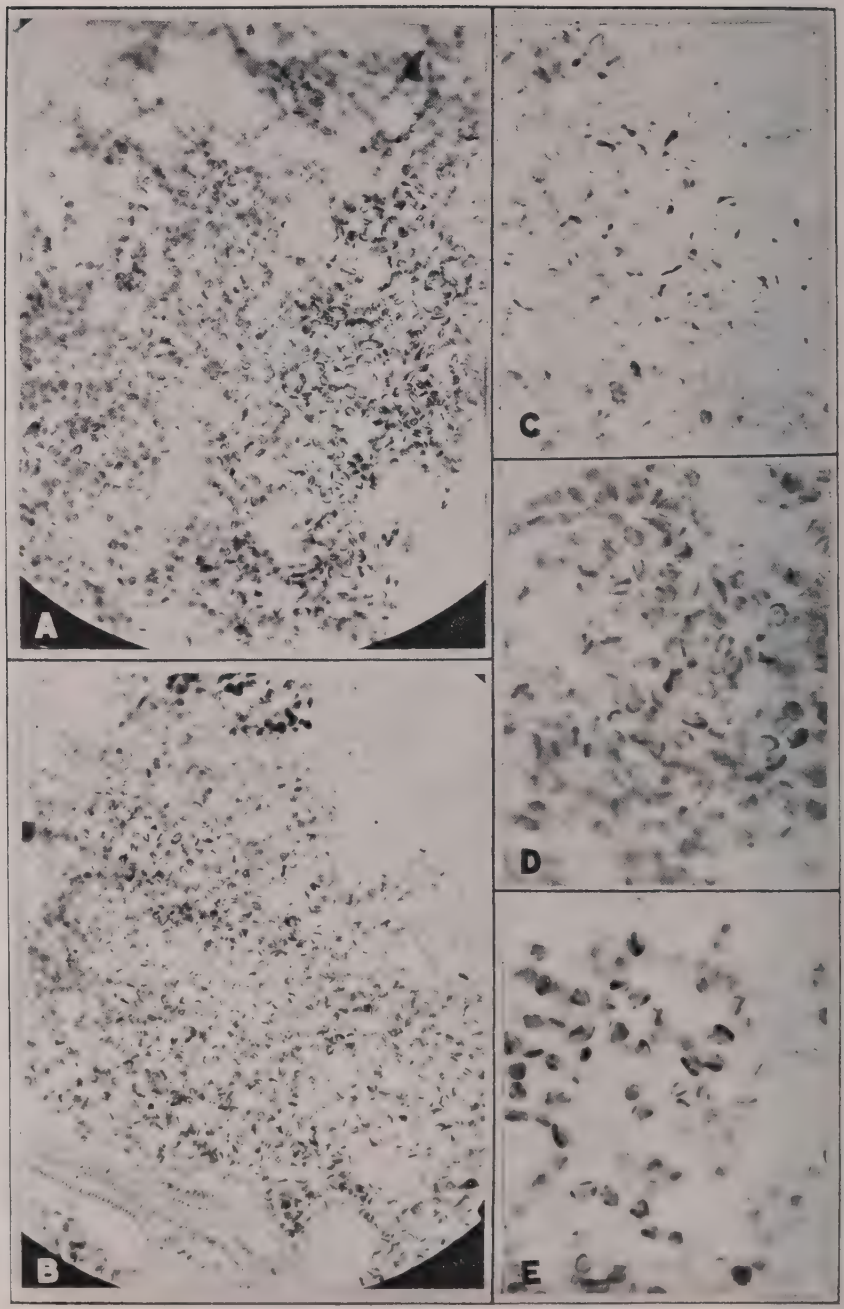


Plate X

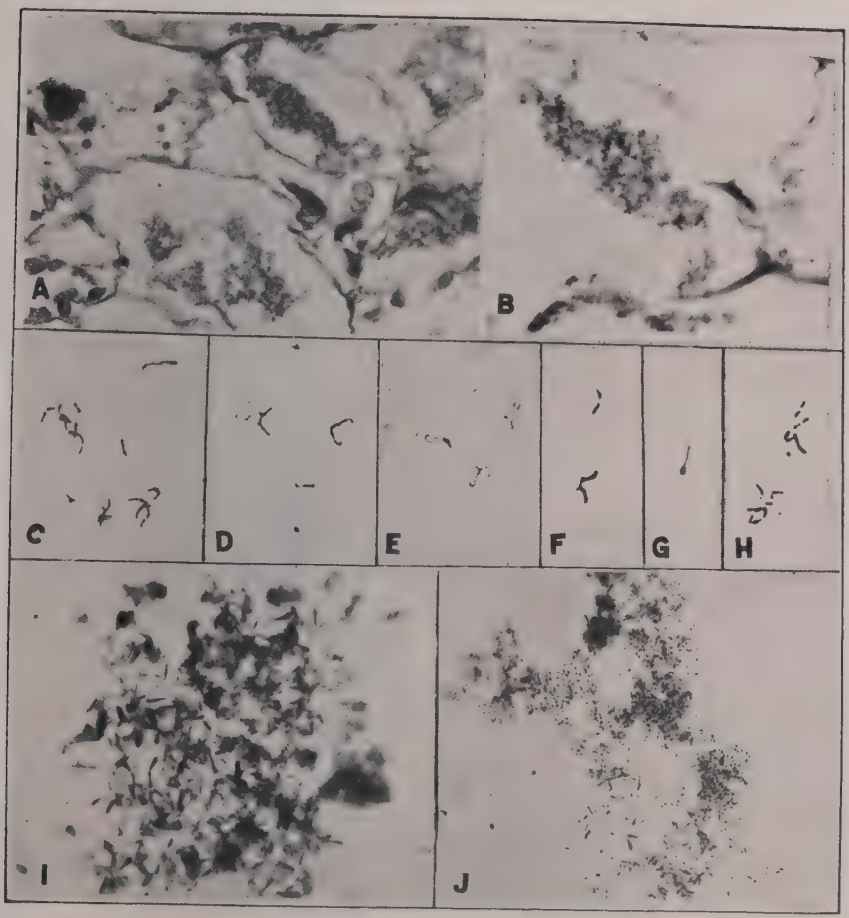


Plate XI

plants. Cultures were made in sterile bouillon from the hypoplastic tissues by sterilizing the leaf surfaces and crushing the tissue in the liquid medium. Growth in the inoculated culture liquid was apparent in two days at room temperature. The organism isolated occurred in zoogleal masses and in *Streptococcus*-like chains. Inoculations were made from the original cultures and infections obtained in 12 of the 15 plants inoculated. Since diseased tissue containing the virus was used for making the cultures, the possibility of dilute virus being present in the liquid medium used for inoculation was not eliminated, a criticism recognized by Dickson as throwing much doubt on the probability of infection being caused by the organism that developed in the culture tubes. It is regrettable that tests were not made to determine the pathogenicity of the organism when grown in remote subplants.

Using filtered sap from mosaic tomato plants as a source of virus and a modified culture medium, consisting of dilute, sterilized extract from the stems and leaves of healthy tomato plants into which was introduced a small piece of sterile tissue from the interior of a tomato fruit, Bewley (1923) claimed to have cultured an organism by anaerobic methods. After two months of incubation, no growth was apparent, but, when the tubes were left in the laboratory for two additional months under aerobic conditions, small, brown bodies, 200 microns in diameter developed on the walls of the tubes above the medium. When stained by the method of Giemsa, these bodies resembled bacterial colonies. The colonies were made up of cocci, diplococci, polar bodies, or unstained rods. Growth was obtained in subcultures made in tomato extract and lemco gelatin. Colonies were transferred to Noguchi tubes and dissolved in the liquid. Films prepared 10 days later from this liquid showed the presence of minute granules, either singly as diplococci or as aggregates in alveolar, plasmodium-like structures, in which cocci stood out deeply stained in comparison with the faintly stained matrix. No conclusive results were obtained in inoculation experiments.

Olitsky (1924) reported the successful cultivation of the virus of tobacco and tomato mosaic in a filtered extract from tomato leaves. Later (1925) he published a detailed account of his experiments in which he states that in 17 attempts he proved that multiplication of the virus had taken place in his cultures at dilutions far beyond the point where the presence of the virus in the cultures could be explained merely as a dilution of the virus present in the original culture. Infection was obtained with all the subplants up to the sixth.

Mulvania (1925), Purdy (1926), and Goldsworthy (1926) repeated Olitsky's experiments without obtaining evidence that the virus multiplied in any of the subplants. Similar results were obtained by Smith (1928) in his experiments on the cultivation of the virus causing Aucuba mosaic of tomato. These conflicting results are not easily explained unless we assume that the culture medium used by Olitsky was not free of virus. Purdy encountered considerable difficulty in preparing an extract of tomato leaves that was free of virus. On the other hand, Olitsky states that his media were carefully tested before use and found non-infectious.

Grainger (1929) was unable to obtain multiplication of the tobacco mosaic virus in a heat sterilized leaf extract or by culturing the virus in a solution containing presumably living chloroplasts.

The most important results have recently been reported by Swezy and Severin (1930) who have cultivated a filterable organism from sugar beets

affected with curly top and also from filtered extracts prepared from the bodies of the specific leaf-hopper, *Eutettix tenellus* (Baker), that transmits the disease. Growth in the special liquid medium used was regularly obtained when it was inoculated with a loopful of the filtrate from diseased beet roots or from the filtered extracts of the salivary glands and intestines of infective hoppers. Growth never occurred in the tubes inoculated with similar extracts prepared from healthy beets and non-infective hoppers. No visible organisms could be demonstrated in either healthy or diseased beet plants, but cytological studies of infective and non-infective insects revealed, in both, organisms similar to those obtained in the cultures. They were found in large numbers in the lumen of the intestines and also as intracellular parasites in the cells of the stomach wall. No organisms were found in the salivary glands but cultures made from these organs taken from infective hoppers were positive. The organisms were demonstrated much more frequently in the infective hoppers than in the non-infective individuals. The organsims in culture and in the insects have morphological characteristics which seem to relate them to the *Rickettsiae*.

The cultivation of a filterable organism from beet plants affected with curly top and also from the specific insect vector should stimulate new interest in the bacteriology of virus disease investigations. The potential importance of the results of this investigation need not be minimized by the finding of morphologically similar but non-filterable organisms in the non-infective leaf-hoppers. The *Rickettsiae* are organisms known to inhabit the intestines of many insects (Cowdry, 1923). All of the diseases known to be caused by these organisms are transmitted by insects and the non-infective individuals also harbor *Rickettsiae* in their intestines. It seems, moreover, fairly well established that the insect vectors carry both parasitic and non-parasitic organisms of this type. The refractory behavior of the parasitic forms to cultivation outside of the host has added to the difficulty of separating these species from the extracellular insect commensals. Further reports on the curly top investigations will be awaited with interest.

It is of considerable interest that some investigators consider the *Rickettsiae* as forms of other organisms engendered under the influence of the bacteriophage or by other means. Thus Fejgin (1929) believes that *Rickettsia prowazeki* is a stage in the life cycle of *Proteus X*. 19, an organism present in the urine of typhus patients.

In concluding a review of the literature pertinent to the bacteriological studies about to be reported, attention should be directed to recent developments in the study of filterable stages in the ontogeny of the bacteria. The question of the existence of such stages in the life histories of the bacteria has been, for some time, a controversial one, the more conservative bacteriologists refusing to accept the evidence that has been presented. It is not necessary to consider here the numerous papers having a bearing on this question. Hadley (1931) discusses the question thoroughly in a recent publication and presents evidence to show that filterable stages of many of the common species of pathogenic bacteria can be induced by various methods. The filterable stage of the Shiga bacillus has been isolated and shown to be very stable in culture. This problem has a direct bearing upon the etiology of diseases caused by filterable organisms and the results that have been obtained by workers in this field should be utilized in the investigations of plant virus disease etiology.

MATERIAL AND METHODS OF THE BACTERIOLOGICAL INVESTIGATION

The bacteriological studies were started in 1927 after cocci had been observed in the disintegrating chloroplasts of mosaic bean plants. Using ordinary media, attempts were made to isolate the organisms from petioles, leaves, and stems after surface sterilization with mercuric chloride. All such attempts were unsuccessful. In 1928, the organism was isolated for the first time from plants grown under sterile conditions, using as a culture medium the natural, unheated juice from the young pods produced on healthy plants. The methods of preparation of this medium will be described in detail.

Preparation of Media

The medium used throughout these investigations has been prepared from young pods of healthy bean plants and consists of the undiluted, clarified juices sterilized by filtration. This medium was first prepared from very young pods bought on the market in February, 1928. The pods were washed thoroughly and ground finely in a food chopper. Most of the juice was expressed by hand through cheesecloth, the residue by means of a hand press. The green, turbulent liquid was then run into 50 cc. Pyrex centrifuge tubes and centrifuged for one hour at 3,000 r.p.m. Sterilization was accomplished by filtration through Berkefeld W candles. It was then dispensed by means of sterile pipettes into test tubes and incubated for 72 hours before use. With this medium, the coccoid organism was isolated from the leaves of mosaic plants grown under sterile conditions.

Extensive investigations were begun in the spring of 1928, and, at the outset, some of the recognized difficulties were the finding of suitable varieties of beans to be used for the preparation of the culture medium and the working out of time and labor saving methods of clarifying and sterilizing the liquid obtained from the pods. A considerable number of bean varieties were grown in the field, including Black Valentine, Green Pod Stringless, Giant Stringless, Full Measure, Refugee, navy pea, and others. The pods from these varieties were harvested at different stages of development and the medium prepared from them tested for desirable characteristics. Considerable variation was found, the most important observation being that only young pods were suitable for use. The tannin content of the pods seems to increase very rapidly after the seeds begin to enlarge, and beyond a certain unavoidable minimum its presence in the medium is highly undesirable. Whether or not it has a bactericidal action, as claimed, is not known but it does cause the medium to darken and become less satisfactory in its physical characteristics. Therefore, where possible, only young pods have been used in preparing the medium. The most suitable age seems to be just after the seeds are formed in the pods. At this stage, the juice is easier to clarify, and, therefore, filters more readily. It is also higher in sugar content. One of the most desirable varieties found so far is Black Valentine. The pods of this variety are not stringy when young, it is susceptible to mosaic, and gives a good yield of pods high in sugar content.

A good commercial strain of Black Valentine was secured and the seed planted at two week intervals, beginning about June 1. The plots were grown under isolated conditions to keep the planting as free as possible from mosaic. Weekly inspections were made and any plants showing mosaic symptoms were immediately removed and destroyed. The pods were harvested every few days after the first ones were large enough for use. They

were washed thoroughly in tap water and rinsed in distilled water, after which the ends were snipped off with scissors. Maceration was accomplished with a sterilized food chopper and the pulp was squeezed, first by hand, in several layers of cheese cloth and finally in a hand press. The dark green liquid was immediately passed through the Sharples centrifuge at 30,000 r.p.m. and again at 40,000 r.p.m. after the sediment had been removed from the bowl. Extensive tests have shown that in most cases the liquid will not be clarified sufficiently by these two passages through the centrifuge and that great difficulty will be encountered in getting it through the filters. This is due to colloidal material that is not thrown out by the centrifugal force. After testing different methods, it was found that storage of the partially clarified juice at a temperature of 1-2° C. for two or three days resulted in a partial aggregation of the gummy material and that two additional passages through the centrifuge at 40,000 r.p.m. would clarify it enough to make filtration comparatively easy. This procedure is recommended where filtration difficulties are encountered.

The clarified liquid is passed immediately through a sterile 5 x 2-inch Berkefeld V candle but no precautions are taken to keep the filtrate sterile. The preliminary filtration through the V candle completes the clarification by the adsorption of the remaining colloidal material to the surface of the filter. In carrying out the sterilizing filtration, a method has been perfected that has eliminated the failures that occurred in some of the earlier filtrations. A special filter flask that permits the dispensing of the contained liquid directly into test tubes and keeps it protected from falling dust particles in the air has been supplied at our request by the Corning Glass Works. These flasks may be obtained on special order and have been used with gratifying results in this investigation. Only thoroughly tested Berkefeld W, 5 x 1 inch candles are used for the final filtration and these are assembled with the mantel and cork wrapped in paper and sterilized in the autoclave for 20 minutes at 20 pounds pressure. Preceding the sterilization, a liter of distilled water is run through to assure a thorough wetting of the candle. The special filter flasks are not taken from the electric oven until the sterile candles are removed from the autoclave. The final assembly is carried out in a bacteriological culture room which is steamed with high pressure steam for one-half hour a short time before it is to be used. The dispensing of the sterilized liquid into the Pyrex test tubes is also done in this room. Care is necessary throughout the entire procedure of preparing the medium but with the method just described no contaminations have occurred in the different lots of culture medium prepared during the past two seasons.

The sterilized culture medium appears to undergo certain changes after long standing so that it is best to prepare the amount needed just before use. Excess amounts of the liquid have been stored in sealed Erlenmeyer flasks at 1° C. for several weeks and have been used successfully. The pH value of the freshly prepared juice is around 6.2 for Black Valentine and this does not appear to change very much during storage at low temperature. When the pods are used at the right stage of development and the medium is prepared as described, it should be a very light amber color in the flasks and should appear practically colorless when examined in the test tubes.

In some of the experiments a solid medium has been desirable and both gelatin and agar as a solidifying material have been tested. Iso-electric gelatin was prepared by the Loeb method (1924). In preparing the agar for use, the desired amount of Bacto agar was washed in a Buchner funnel with 25 liters of warm distilled water to free it of alkaline salts. These

gels have been used as a base to which the bean pod juice was added just before needed. The gel was melted and cooled to 40° C. and the liquid from the special filter flask added in the desired amount to each tube. One-quarter per cent agar and 10 per cent gelatin were used in preparing semi-solid media for some of the cultivation tests. As possible adjuvants to the sterile bean pod juice, the washed agar and isoelectric gelatin added in quantities to make a semi-solid medium have not yet been given a very extensive test.

Beef infusion has been used as a medium for subculturing the cocci after they have become adapted to growth in an artificial medium. After several subplants in the bean pod juice, they can be subcultured in the beef infusion. This medium was prepared from the best grade of lean beef and Difco peptone. No adjustment of the reaction was made, the pH of the liquid being approximately that of the natural bean juice.

Material for the Isolation Experiments

The first successful isolations of the cocci were made from mosaic bean plants grown under sterile conditions. Pods were removed from mosaic plants when the seeds were mature but before there was any evidence of dehiscence. The surface of the pods was sterilized with mercuric chloride and the seeds removed with aseptic precautions to sterile test tubes. After they had become dry, they were placed on the surface of agar in petri dishes to test for surface sterility. If no contaminants appeared, the seeds were transferred to a suitable substratum in large sterile test tubes (Plate III, B). Either soil or a synthetic solution incorporated in agar can be used; the latter is more easily sterilized. By this method sterile plants can be produced showing typical symptoms of mosaic (Plate III, C). It is not easy to keep the interior of the tubes sterile for a long period of time and the use of this method was obviated after it was found that the seeds from young pods could be used as a source of mosaic-infected tissues.

It has been shown in the seed transmission studies that the virus of bean mosaic is transmitted to approximately one-half of the seeds from a population of diseased plants. The virus is contained in some portion of the young seeds and cultures can be made directly from these tissues, which are, as will be shown, free from the ordinary saprophytic organisms. This method of making cultures from mosaic and mosaic-free plants has been used almost entirely in these investigations.

Technic Employed in Making Isolations

All of the isolation and subculture work has been carried out in a special bacteriological culture room equipped with high pressure steam pipes so that the walls and air might be comparatively freed of air-borne organisms. This was accomplished by steaming the room for 30 or more minutes an hour or so before it was to be used. In making the cultures from the plants grown in the large test tubes, the outside of the tubes and the cotton plugs were first sprayed with 95 per cent alcohol which was then ignited before the tubes were taken into the culture room. After a thorough flaming of the plug and mouth of the tube, the portions of the plants to be used were severed with sterile scissors and placed in sterile petri dishes. The cultures were then made by crushing small pieces of the tissues in tubes containing the sterile bean pod juice.

In making isolations from infected seeds, the pods are usually taken from

plants that have been grown from diseased seed. This procedure is based on the assumption that in such pods the virus will have had the best chance to reach at least some of the seeds, although it has been shown in the seed transmission tests of the disease that occasionally all of the seeds from some plants fail to receive the virus. The pods are removed from the plant just before they are to be used, soaked for 5 to 10 minutes in mercuric chloride, rinsed in sterile water, and transferred to sterile dishes, all of the pods from each plant being placed in one dish. The pods from each plant are given consecutive numbers as they are used and the number of the pod and the position of the seed in the pod is recorded on the test tube. The pods are torn open along the sutures with forceps and the seeds carefully removed with sterile forceps, beginning with number 1 at the apical end. Each seed as it is removed is transferred to the thoroughly flamed mouth of the test tube and crushed in the liquid medium with small, sterile, steel scalpels.

The opportunities for contaminations to occur have been reduced to a minimum by the methods used. Freshly laundered, long white surgeons coats have always been worn during the isolation and subculture work; and, to eliminate the possible objection that cocci from the respiratory passages might contaminate the cultures, control tests have been made with a respirometer constructed from sterile gauze. The tests have shown that this is not a valid objection.

The cultures have been incubated at room temperature or at 25° C. in an electric incubator. The first examination of the cultures has always been made after 24 hours of incubation. Gross contaminations are usually evident at this time and the growth of contaminating organisms is observable in the tubes after a period rarely exceeding 36 hours from the time the isolations are made, but growth of the coccoid organisms can seldom, if ever, be detected in the tubes prior to 72 hours after the isolations are made and very frequently is delayed until the sixth day. The cultures are examined at 24-hour intervals by means of strong transmitted light against a black background. By this method, the faint characteristic clouding of the cultures containing the coccoid organisms in the chain form can usually be detected. In the single and diplo forms, the cocci produce a heavier growth and the clouding of the medium is more dense. Colloidal materials from the crushed seeds cause varying degrees of opacity in the liquid and examination by strong transmitted light against a black background is necessary to distinguish this clouding from that due to the growth of the organisms. By slightly agitating the liquid and giving it a swirling motion, the clouding due to the organisms can be readily distinguished from that due to smaller colloidal particles. If no visible growth has occurred in the tubes by the sixth to eighth day, they are discarded.

Microscopic examination of the cultures has been made in hanging drops, and films stained with Giemsa stain have been prepared in the case of all doubtful tubes. This is especially important with the tubes showing the delicate growth of the extremely small, chain forms of the cocci and also with the Rickettsial forms that have been isolated from rugose mosaic plants.

Subcultures have been made as soon as possible after the microscopic examination. The organisms are extremely sensitive to aging, especially in the original cultures, and unless subplants are made within two to five days after visible evidence of growth is seen, failure of the organisms to grow in the subplants is likely to occur. Subculturing of the organisms at five to seven day intervals is essential for their continued growth. In making the subplants, it has been found advantageous to use sterile pipettes instead of

platinum loops, since one of the essential factors in securing growth in the subculture tubes seems to be the seeding of the medium with one-tenth to one-quarter of 1 cc. of the liquid containing the organism. Frequently, it has been necessary to resort to the use of tissue cultures in order to obtain growth in some of the tubes, especially with the pleomorphic organism from rugose mosaic plants and the minute streptococcal forms, each of which has never been grown without the tissue as an adjuvant. The tissue cultures have been prepared by removing from healthy plants pods containing immature seeds. The pods are sterilized in mercuric chloride and the seeds removed with aseptic precautions to sterile petri dishes. Each seed is cut into two equal parts with a sterile scalpel and the cut surface of each half sealed by dipping it in melted plain agar at a temperature of about 45° C. This prevents starch and colloidal materials from diffusing into and causing a clouding of the liquid. Apparently, it does not prevent the outward diffusion of nutrient or hormonal substances which increase the growth-supporting qualities of the medium. The tissue may decrease the oxygen or increase the CO_2 tension in the liquid and thus aid in stimulating growth of the organisms refractory to cultivation in the plain broth. In any case, the use of the tissue has proved indispensable in the cultivation of forms of the organisms that could not be grown in the plain liquid medium.

RESULTS OF THE ISOLATION EXPERIMENTS

Cocci were first isolated from the tissues of mosaic bean plants in March, 1928. Mosaic plants growing under sterile conditions in the large test tubes furnished the infected tissue for the first successful isolation tests. Pieces of tissue were excised from mottled and from normal-appearing leaflets and placed in petri dishes containing 10 cc. of the bean pod juice solidified with agar. The petri dishes were placed in sterilized Novy jars and a portion of the air in the jars displaced with filtered oxygen. More oxygen was added at 12-hour intervals and on the third day the jars were opened and the dishes examined with a low power lens. Surrounding some of the pieces of tissue from the mottled leaflets, there was observed a faint opalescent haze. These pieces of tissue were transferred directly to plain bean pod juice in ordinary test tubes and also to the same medium in larger tubes equipped so that filtered oxygen gas could be bubbled slowly through the liquid. This latter procedure was based on the assumption that a requirement for the multiplication of the organisms might possibly be increased oxygen tension. Since the organism had been observed in the chloroplasts, where, during assimilation, the oxygen tension is in all probability much higher than in any other portion of the cell, it was thought that by using oxygen as a stimulant the organism could be grown in vitro. Consequently, oxygen was used to displace part of the air in the Novy jars. It was filtered thoroughly by passing it through glass tubing filled with sterile absorbent cotton and bubbled slowly through some of the tubes containing the pieces of tissue. The method previously had been tested with tubes of plain bean pod juice, and at the rate of flow with which the gas was bubbled through the liquid, no bacterial growth occurred in any of the control tubes. The sterile glass tubing extending to the bottom of the culture tubes was drawn out to a capillary fine point and the oxygen was thus released in very small bubbles.

Growth occurred in the oxygenated medium containing the pieces of tissue, around which an opalescent zone of haze had developed during incubation in the Novy jars, but in the tubes that were not oxygenated no clouding of

the medium occurred. No growth occurred in any of the tubes containing the tissue from the normal plants. An examination of the cultures in hanging drops showed the presence of a small coccus in all of the tubes in which clouding occurred. From these results, it was thought that perhaps the oxygen was indispensable for the isolation of the organism but subsequent results demonstrated the error of this view, and, following the isolation of the coccus by other methods, it was not used further.

A simpler method of isolating the cocci from mosaic plants was developed during the spring and summer of 1928 but it was not given an extensive test until 1929. The method was first used in April, 1928, with Refugee plants affected with rugose mosaic. Isolations of a very pleomorphic bacterial organism were made from the young seeds which were removed with aseptic precautions from the pods, the surface of which previously had been sterilized with mercuric chloride. The success of this experiment in developing a short and convenient method which could be used instead of the unsatisfactory and tedious one of growing plants under sterile conditions was immediately followed by its application to the isolation work with the true mosaic disease. Cocci were obtained from a number of mosaic plants during the summer of 1928 by means of isolations from young seeds taken with aseptic precautions from disinfected pods, but difficulties in preparing a suitable medium were not overcome until the end of the growing season and the method was not given a complete test. During 1929, however, isolations were made from a large number of field-grown plants. A large quantity of the pod extract had been prepared in July and August from Black Valentine plants and on August 23 the first isolations were made from 10 pods taken from 10 mosaic navy pea bean plants. Thirty-two cultures were made from the young seeds in the 10 pods. After a 72 hour incubation period, 13 of the tubes showed clouding and at the end of the fifth day two more tubes also showed evidence of bacterial growth by clouding of the liquid. Cocci exclusively, were present in all of the 15 tubes; in 13 they were present in the paired or diplo form and in the remaining two they occurred in long delicate chains. The remaining 17 tubes showed no indications of any change in the medium and no clouding of the liquid could be detected in any of them. In this experiment, 46 per cent of the seeds from 10 pods were infected with cocci. The ratio of seed infection by the cocci is similar to that of seed infection by the virus and the results of this experiment first suggested a possible relationship between the presence of the virus and the cocci in the seeds from mosaic plants. This was tested more thoroughly in the 1930 experiments. The spread of mosaic into the field plantings of beans grown from non-infected seed, spoiled these plants for isolation work and plantings of the susceptible variety, Black Valentine, were made in the greenhouse so that pods from which isolations could be made at a later date would be available.

Additional cultures were made from mosaic navy pea bean plants on October 1. Eighteen pods containing immature seed were disinfected; the seeds were removed aseptically and used for inoculating tubes of the pod extract. The results of this test are given in Table V. The majority of the plants from which the pods were taken had become infected during the current growing season, some being early and some late infections.

The summarized results of this test show that of the 34 young seeds taken from the 18 pods, nine were infected with cocci. One culture showed heavy clouding in 24 hours due to the presence of a large spore-forming bacillus, an unquestionable accidental contaminant. Approximately 27 per cent of the

Table V.—Isolations from young seeds of mosaic navy pea beans October 1, 1929. Records October 6.

Pod number	Seed	Culture record	Pod number	Seed	Culture record
1	1*	—	9	1*	—
2	1	Coccus	10	1	—
	2	—		2	Coccus in chains
	3	Coccus	11	1	—
3	1	—		2	—
	2	—	12	1*	—
4	1	—	13	1	—
	2	Coccus		2	—
	3	—		3	—
5	1	—	14	1*	—
	2	Contaminated	15	1*	—
	3	—	16	1	Coccus
	4	Coccus		2	—
6	1*	—	17	1	Coccus
7	1	Coccus		2	—
	2	Coccus	18	1*	—
8	1	—			
	2	—			

*Single seed in the pod.

seeds were infected, a ratio comparing very well with the known facts concerning the transmission of the virus to seeds in plants becoming infected during the current growing season.

Isolations were next made from the apparently healthy Black Valentine plants growing in the greenhouse. The seed had been planted August 28 and the plants were tall and strongly indeterminate in their growth. This same strain had been used for the field plantings to produce pods as a source of extract for the bacteriological work. About 2 per cent of the seeds were infected with mosaic, and, in the field, this is easily detected and is rogued out as soon as the first trifoliate leaf is produced. In the greenhouse, in the fall and winter plantings the plant grows very tall and spindly and mosaic symptoms are difficult to recognize. No attempts had been made to rogue any plants from this particular planting and the isolations were made on October 25 from pods selected at random from a number of plants. Eleven pods containing a total of 32 young seeds were used in the test. From the 32 seeds, five cultures of micrococci were obtained. The isolation of a coccoid organism from apparently normal Black Valentine plants raises the question as to whether or not normal appearing plants are infected with an organism similar to the cocci isolated from mosaic plants. No decisive answer to this question can be given because of the inconsistent results that have been obtained in the isolation tests from the young seeds of field-grown plants of highly susceptible varieties like the Stringless Refugee. In a few cases, cocci have been isolated from Refugee plants apparently free from symptoms of mosaic. These have always been obtained from plants grown in the open and in plantings which developed mosaic through insect transference of the virus. Plants of susceptible varieties grown in the greenhouse or in the field and protected from insects have always given only sterile cultures from the seeds. By the method of growing plants in large test tubes under sterile conditions, evidence has been obtained that cocci are not present in the tissues of these plants.

Seeds of three very susceptible varieties of beans were planted on August 28 in the large test tubes and plants of each in the varieties grown under sterile conditions. The seeds from which these sterile plants were obtained had been

harvested under aseptic conditions during the summer months from pot-grown plants and stored in sterile test tubes. The tubes containing the plants were kept in a small, ventilated cage which was lighted with a single 1,000 watt mazda lamp to stimulate growth and intensify symptoms if mosaic might perchance be carried in any of the seeds. All of the plants produced in the tubes were apparently normal and showed no evidence of disease. The isolations were made, by the method that has been described, from pieces of tissue of three Black Valentine, three navy pea, and two Stringless Refugee plants. The results are given in Table VI.

Table VI.—Isolations from apparently normal mosaic-susceptible bean plants grown under sterile conditions. Dec. 12, 1929.

Variety	Plant No.	Cultures	Tissue used for Inoculating Culture Medium	Culture record
Black Valentine	1	5	Stem, all levels	All negative
		4	Leaflets	All negative
	2	2	Petiole	1 neg., 1 large bacillus
		4	Leaflets	3 neg., 1 yeast
	3	2	Petiole	All negative
		4	Stem	All negative
Navy Pea	1	2	Petioles	All negative
		2	Stem	All negative
	2	3	Leaflets	All negative
		2	Petiole	All negative
	3	4	Stem	All negative
Stringless	1	4	Stem	All negative
Refugee	2	3	Petioles	All negative

Similar experiments with mosaic-free navy pea beans had been conducted in 1928 at Ann Arbor and no bacteria were isolated from the tissues of such plants. These tests have shown that plants grown under sterile conditions do not contain in their tissues bacteria that can be cultured by this method. From mosaic plants grown under the same conditions, cocci have been isolated but the percentage of successful cultures obtained by this method has always been lower than that from isolations made from the young seeds. In a number of cases, the isolations made from mosaic plants grown in the tubes have not yielded cultures of the cocci. This may possibly be explained on the basis of the localized distribution of the organism in the plant but a similar reasoning might be used to explain the failure to obtain cocci in the isolations made from apparently normal plants. The 1930 experiments, however, strengthen the evidence that mosaic-free plants are not, in the majority of cases, infected with coccoid bacteria but that these organisms do occur regularly in mosaic plants.

Much more extensive experiments were conducted during the season of 1930. More than 400 cultures were made from field-grown bean plants of a number of susceptible varieties, including navy pea, Refugee, Black Valentine, Great Northern, and others. Some plants of the resistant variety Robust were included. Seeds of the various varieties were planted about the middle of June, the mosaic-infected seed in one plot and the non-infected seed in another plot about 50 rods distant. Plants of some of the varieties were inclosed in long cheesecloth cages to exclude insects. The use of these cages was an especially fortunate thing, for due to the extreme drought conditions and the high temperatures that characterized the season of 1930, some of the uncaged varieties failed to produce pods. The Great Northern

plants were so dwarfed and stunted that they bore no resemblance to normal plants of this variety. In the cages, however, this variety grew well and produced a good crop of pods. The infected seeds of the Refugee and navy pea varieties were planted in two separate plots, each watered by overhead irrigation. In the Refugee plot, all plants failing to show symptoms of mosaic in the first trifoliate leaf were removed, leaving only plants infected from seed. In the navy pea plot, only plants infected during the current season were used. These plants were tagged as soon as the symptoms developed and the pods taken only from the plants for which this record was available. Most of the plants except those in the cages did not grow normally, pod formation was much delayed, and there was a reduction in number due to the shedding of blossoms from the plants because of hot, drying winds.

Isolations were made on September 23 from the cage-grown Great Northern and Robust plants and from the field-grown uncaged Refugee plants. All of these plants were free from symptoms of mosaic. The Refugee plants were grown close to the cages, were partially shaded, and produced a good set of pods. The results of these isolations are shown in Table VII.

Table VII.—Isolations from apparently healthy bean plants, Sept. 23, 1930. Culture records taken Sept. 28.

Plant	Pod	Seed	Culture Record	Plant	Pod	Seed	Culture Record	Plant	Pod	Seed	Culture Record
Great Northern cage grown plants:				Robust cage-grown:				Refugee, field-grown:			
1	1	2	—	1	1	1	—	1	1	1	—
	2	1	—			2	—			2	—
		3	—			3	—			3	—
		5	—			4	—		2	1	—
	3	1	—			5	—			2	—
		2	—		2	1	—			3	—
		3	—			2	—	2	1	1	—
		4	—			3	—			2	—
		5	—			4	—			3	—
2	1	1	—	2	1	1	—		2	1	—
		2	—			2	—			2	—
		3	—			3	—			3	—
		4	—			4	—	3	1	1	—
	2	1	—		2	1	—			2	Bacillus
		2	—			2	—			3	—
		4	—			3	—		2	1	—
3	1	1	—			4	—			2	—
		2	—	3	1	1	—			3	—
		3	—			2	—	4	1	1	—
		4	—			3	—			2	—
		5	—			4	—	5	1	1	—
	2	1	—		2	1	—			2	—
		2	—			2	—			3	—
		3	—			3	—			4	—
	3	1	—			4	—			5	—
		2	—	4	1	1	—		2	1	—
		3	—			2	—			2	—
		4	—			4	—			3	—
					2	1	—				
						2	—				
						3	—				
						4	—				
						5	—				
				5	1	1	—				
						2	—				
						3	—				
						4	—				
					2	1	—				
						2	—				
						3	—				

Of the 96 cultures made from the three varieties of mosaic-free bean plants, only one showed any evidence of bacterial growth. Clouding of the medium in this tube occurred within 24 hours, and examination in hanging drop showed that it was due to large, motile rods. The medium in 95 of the tubes was unchanged in appearance at the end of the sixth day of incubation at 25° C. The results of this experiment do not lend support to the view that cocci are regularly present in the tissues of normal bean plants. That they may sometimes be present in field-grown plants of some varieties like Black Valentine, Refugee, and navy pea cannot be denied. Neither can it be denied that we have no unfailing criterion by which we can determine whether or not a plant is entirely free from disease when it is grown in the open, freely exposed to the attack of aphids and other vectors of virus diseases.

Isolations were also made on September 23 from the mosaic Refugee and navy pea bean plants. Plants of these two varieties had been grown under irrigation and were not seriously affected by the drought conditions. The Refugee plants were produced from infected seed; all plants that had not shown symptoms of mosaic in the first compound leaf had been rogued out in June. Accordingly, the plants from which the isolations were made had been diseased throughout the entire growing season. Only pods containing young seeds were selected and these were taken from 10 plants showing typical symptoms of mosaic. The results of the isolations from these 10 plants are shown in Table VIII.

Table VIII.—Isolations from mosaic Refugee bean plants, Sept. 23, 1930. Culture records Sept. 28.

Plant	Pod	Seed	Culture Record	Plant	Pod	Seed	Culture Record	Plant	Pod	Seed	Culture Record
1	1	1	Coccus	4	1	1	—	7	1	1	Coccus
		2	Coccus			2	—			2	—
		3	Coccus			3	—			3	Coccus
	2	1	Coccus		2	1	—			4	Coccus
		2	Coccus			2	—			5	Coccus
		3	Coccus			3	—	8	1	1	—
2	1	1	Coccus	5	1	1	—			2	—
		2	Coccus			2	—			3	—
		3	Coccus			3	—			4	—
		4	—			4	—		2	1	Coccus
	2	1	Coccus		2	1	Coccus			2	Coccus
		2	Coccus			2	Coccus			3	Coccus
		3	Coccus			3	Coccus			4	Rickettsia
3	1	1	—	6	1	1	Coccus			5	—
		2	—			3	Coccus	9	1	1	—
		3	—			5	Coccus			3	—
		4	—						2	1	Coccus
									3	1	Coccus
								10	2	1	—
										2	—
										3	Motile rods
										5	Coccus
										6	—

Twenty-seven, 48 per cent, of the 56 seeds from the mosaic Refugee plants were infected with the coccus. The organism in one culture agreed in all of its morphological characteristics and reaction to stains with the Rickettsiae. One culture had apparently been contaminated, for motile rods clouded the medium on the second day of incubation. The per cent of the seeds infected with the coccus agrees closely with the per cent of seed transmission of the virus in plants which have been diseased throughout the

growing season. If the seeds from which the isolations were made had been allowed to mature, it is probable that approximately half of them would have given rise to mosaic seedlings. Since the evidence seems to indicate that the virus must reach the ovule before or shortly after fertilization in order to cause infection, no further increase in the number of infected seeds could be expected during the additional time required for the seeds to mature.

It has been shown repeatedly in seed transmission studies that if seeds are harvested from a large number of primary-infected mosaic plants about half of the seedlings will be affected with the disease. The correlation between the presence of the virus and the coccus seems to be fairly constant if we can rely on the isolation experiments as a criterion. The close relationship between virus and coccus is still further brought out in the isolations made from the navy pea plants. All of the plants from which the isolations were made had become infected during the current season. In seeds harvested from mosaic plants that become infected during the growing season, the per cent of transmission of the disease is always smaller than in seeds taken from plants grown from infected seed. The per cent of mosaic transmission through the seeds from seasonally infected plants varies considerably, depending very likely upon several factors, one of which is the time that infection occurs. The longer the plant is infected previous to the flowering period the greater will be the opportunity for the seeds to become infected. Plants infected after the flowering period do not, as a general rule, transmit mosaic through the seed. The seeds of the navy pea variety had been planted about June 25, and symptoms of mosaic were first noticed in the leaflets on July 27 when the plants were tagged. Infection had very likely occurred sometime between the first and second compound leaf stage. Isolations were made from 17 pods taken from nine typically diseased plants. In Table IX are shown the results of the isolations made from each seed.

Table IX.—Isolations from navy pea bean plants infected with mosaic during the current growing season. Sept. 23, 1930. Culture records Sept. 28.

Plant	Pod	Seed	Culture Record	Plant	Pod	Seed	Culture Record	Plant	Pod	Seed	Culture Record
1	1	1	—	4	1	1	—	7	1	1	—
		2	—			2	—			3	—
		3	—		2	1	Coccus			4	—
		4	—			2	—			5	—
		5	—			3	—		2	1	—
	2	1	—	5	1	1	—			2	—
		2	Coccus			2	—			3	—
		3	—			3	—			4	—
2	1	1	—			4	—	8	1	1	—
		3	—			5	—			2	—
	2	1	—		2	1	—		2	1	Coccus
		2	—			2	—			2	Coccus
		3	—	6	1	1	Coccus			3	Coccus
3	1	1	—			2	—	9	1	1	—
		3	—			3	Coccus			2	—
		4	—								
		5	—								
	2	1	—								
		2	Bacillus								
		3	—								
		4	Coccus								

Of the 56 cultures made from the nine plants infected during the current season, eight, 14.2 per cent, developed clouding in the medium due to the growth of cocci. Clouding developed in 24 hours in one tube due to the growth of a large bacillus. In the remaining 47 tubes, no clouding of the medium had developed at the end of the sixth day when the final readings were made.

Again, in the isolations from these seasonally infected plants is seen a close correlation between the rate of seed transmission of the virus in plants infected during the current growing season and the presence of the coccus in the young seeds produced on these plants. Obviously, the limitations of the method do not permit the establishment of absolute proof that the coccus is present in all of the seeds which transmit mosaic to the seedlings. Seedling from an infected seed can not be grown and isolations made from that same seed with the technique that has been used in these investigations. The location of the virus in the seed is not known; neither is it known at present in which portion of the seed the coccus may be found. The solution of the latter problem will be much less difficult than a determination of the tissues infected by the virus. In the present investigations, it has been established that cocci are regularly present in the seeds of mosaic-infected plants, and that the results of the bacteriological studies in connection with the results of the seed transmission tests indicate that in these diseased plants there is a close association of the coccus and the virus, whatever the latter may be.

RUGOSE MOSAIC

HISTORY AND DESCRIPTION OF SYMPTOMS

During the summer of 1927, there were observed among a large planting of beans, including crosses between various varieties, some plants in one variety that showed a very severe crinkling of the foliage. The crinkling was observed first in the simple leaves of some of the plants and later it developed more severely in the succeeding compound leaves. Approximately one-half of the plants in this particular row were affected in this way. The leaves were dark green in color and unmottled but presented such a conspicuous and contrasting appearance to those of the other plants in this collection that attention was immediately centered upon them. The plants were observed throughout the growing season, leaf and petiole material was collected for cytological study and in September seed was harvested from a number of plants that had been staked during the summer.

The disease has been under observation during four growing seasons in the field and affected plants have been grown through repeated generations in the greenhouse. During this period, the symptoms have always been the same as those in the original plants and the causal agent has been transmitted through the seed in the same ratio as the true mosaic. It seems certain that this disease is a virus trouble and on the basis of symptoms it appears to be distinct from the true mosaic which has been compared with it on the same variety of bean. It has been observed only on the Stringless Refugee variety and aside from its occurrence in this 1927 planting, has been found only once elsewhere. One plant affected with this disease was observed in a planting of Stringless Refugee beans at Salinas, California, in September, 1929.

The leaflets of severely affected plants are usually dark green in color and resemble the laminae of savoy cabbage plants (Plate V, A). The savoyed appearance of diseased leaves is the most characteristic field symptom and rarely may be apparent in the simple leaves but usually does not become evident until the first compound leaf has developed. The crinkling and curling of the foliage is variable in its severity on plants grown under different conditions. In plants grown in the greenhouse during the winter months, only a moderate ruffling of the lamina of the leaflets may develop. During the spring, this increases in severity and by April greenhouse-grown plants show a characteristic crinkling that is less severe than is shown by plants grown in the field. Sometimes the pods are affected (Plate V, B) developing a ruffling of the tissue that may become evident at an early stage in their development. The seed transmission studies definitely relate this trouble to the mosaic diseases and a study of the constancy of symptoms adds additional weight to this probability. During the winter months, diseased plants develop a yellow mosaic mottling particularly in the tissues at the bases of the leaflets, and the entire plant may become somewhat chlorotic, symptoms which are not observable in the field. From the observations that have been made of affected plants, especially the differences shown by Stringless Refugee plants affected with this trouble in comparison with other plants of this same variety affected with the true mosaic disease, there seems sufficient reason for describing it, tentatively at least, as a distinct disease. It has not been transmitted by artificial means, and, until extensive transmission studies have been conducted with a number of varieties, a final classification should be deferred. The name rugose mosaic is suggested for the disease.

BACTERIOLOGICAL INVESTIGATIONS

In April, 1928, isolations were made from young seeds of greenhouse-grown plants. The methods that have been described in the foregoing sections were used throughout the investigation of rugose mosaic. The original isolations were made with the aid of oxygen. The young seeds were removed with aseptic precautions to sterile petri dishes and each seed was cut into equal halves with a sterile scalpel. One-half of each seed was planted immediately without crushing in the bean pod juice, the other half was placed cut surface downward upon the surface of the bean juice agar in sterile petri dishes. The petri dishes were then placed in sterile Novy jars and a portion of the air in the jar displaced with pure, filtered oxygen. An additional quantity of oxygen was added every 12 hours. After 48 hours incubation in the Novy jars, the petri dishes were taken into the culture room and the halves of the seeds removed from the agar surface and placed in tubes of the bean pod juice. The tubes were then incubated at room temperature. At the end of the fourth day, a very faint clouding was visible in some of the tubes. It was necessary to view the liquid in the tubes with strong transmitted light against a black background in order to detect the clouding of the medium. Subplantings to bean pod juice and also to the tissue culture tubes previously described were made immediately and films were strained with Giemsa stain. An examination of the stained films revealed the presence of an organism with very pleomorphic and striking characteristics. The organism bore no resemblance to any known forms of bacteria and was not recognized as such until after the subplants had been carried for some time.

The subplants in the plain bean pod juice remained apparently sterile and

at no time was it possible to obtain evidence of clouding in tubes heavily seeded with the organism from tissue-culture tubes. In the subplants containing the tissue, a faint opalescence was visible after five days of incubation. Viewed by ordinary light against the sky or a light-colored background, no clouding could be detected. The organism grew very feebly in the tubes containing the tissue but it was maintained in subplants, made at seven-day intervals, for a period of 10 weeks. During this time, the organism passed through a cycle of morphological development which included branching rods, balloon forms, rods of many bizarre shapes (some enormously swollen and containing spherical, deep-staining bodies), Rickettsia-like bodies and a coccoid stage (Plate XII, D-J). The development of the organism in the subplants was followed by means of cover slip smears stained with Giemsa stain. With the appearance in the cultures of a very small coccoid stage, the organism failed to grow in the tissue cultures and also in a variety of other media tested in an attempt to revive growth in the subplants. The isolations were made on April 23 and the last subplantings in which growth of the organism occurred were made on June 29. These cultures were not discarded but were kept in the laboratory at room temperature until September. On September 23, subplantings were made again from the June 29 cultures and in the tubes containing tissue a feeble growth occurred. Stained films showed forms of the organisms similar to those that appeared in the original cultures. The organism was kept alive by frequent subculturnig until November 9, when the subplants again failed to show any evidence of activity of the organism. No growth occurred in any of the subplants made at intervals after this date and the cultures were finally discarded after the liquid had completely evaporated from the tubes.

Isolations have subsequently been made from a large number of Refugee plants affected with Rugose mosaic but the pleomorphic organism has not again been cultured from the young seeds. Organisms with a morphology similar to some of the stages that develop in the original cultures have been obtained in many of the cultures. There has been no difficulty in isolating cocci from the seeds of these plants and in the 1930 experiments Rickettsia-like organisms were cultured from field-grown plants. Both a coccoid and a Rickettsial stage developed in the subplants of the cultures obtained in April, 1928. It seems very likely that the organism isolated at that time was cultured during a period when it was undergoing ontogenetic development with the production of the various morphological forms that constitute the complete cycle. The cytological observations furnish evidence that this is more than a speculative possibility for similar forms have been observed in the chloroplasts of diseased plants.

Most of the isolations made previous to 1930 were from plants grown in the greenhouse. In the 1930 experiments, the plants were grown in the field and fortunately the seed was planted in a plot that could be watered by means of overhead irrigation equipment. A count was made of the normal and diseased plants that grew from the seed and the ratio of diseased to normal-appearing plants was practically six to four. The normal plants were removed at the time the second trifoliate leaf appeared. Isolations were made from 77 seeds taken from 39 pods on 13 plants. Many of the pods contained only a single seed, and in others seeds in various loci were aborted. The cultures were made September 25 and were examined on September 28 and October 1. Table X shows the results of these isolations.

Table X.—Isolations from field-grown Refugee plants affected with rugose mosaic Sept. 25, 1930. Culture readings Sept. 28-Oct. 1.

Plant	Pod	Seed	Culture Record	Plant	Pod	Seed	Culture Record	Plant	Pod	Seed	Culture Record
1.....	1	3	—	5.....	1	1*	—	9.....	1	1	Coccus
		4	Rickettsia		2	1	Coccus			2	Coccus
	2	1*	—			2	—			3	—
	3	2	Coccus			3	Coccus		2	1	—
		3	—		3	1	Coccus			2	—
2.....	1	1	—			3	—			3	—
		2	Coccus	6.....	1	1*	Coccus			4	—
	2	1	—		2	2	—	10.....	1	2	Coccus
		2	—			3	—			3	Coccus
		3	Coccus			4	—	11.....	1	1	—
	3	1	Rickettsia		3	1	Motile rod			2	—
		2	—			2	Coccus		2	2	—
3.....	1	1*	Rickettsia	7.....	1	1*	Coccus			3	Coccus
	2	1	Rickettsia		2	1*	Coccus	12.....	1	1	Coccus
		2	Rickettsia		3	1*	Coccus			4	Coccus
		3	Rickettsia		4	1	Coccus		2	1	—
		4	Rickettsia			2	Coccus			2	—
	3	1	—		5	1	—	13.....	1	1	—
		2	Coccus			2	Motile rod		2	2	—
4.....	1	2	—	8.....	1	1*	Rickettsia			4	—
		3	Coccus		2	1*	Rickettsia		3	1	—
	2	1	—		3	1	Rickettsia			2	—
		3	Coccus			3	Rickettsia			3	—
					4	1	—			4	Coccus
						3	—			5	—
						4	—				
					5	1*	—				
					6	1	Coccus				
						3	Coccus				

*Single seed in the pod.

Twenty-six cultures of cocci and 11 of an organism resembling in all morphological details the *Rickettsiae* were obtained from the 77 seeds. Two cultures of a large motile bacillus and 38 tubes showing no change in the medium completed the series. A study of the cultures of the Rickettsia-like organism was made in hanging drops and in films stained by the method of Giemsa. The organism was extremely small, occurring as rods showing bipolar staining or diplococcoid forms. All attempts to grow the organism in subplants both with and without tissue were unsuccessful. Organisms similar to these had previously been noted in some of the cultures made from roguse mosaic plants but had not been subcultured. No doubt this organism was obtained in other cultures and was overlooked due to the delicate growth produced in the medium used. It is interesting to note the similarity of the symptoms of rugose mosaic on the Refugee variety to the symptoms of curly top on beans. Carsner (1926) found that beans were susceptible to curly top and the symptoms as described and pictured by him are very similar to those of rugose mosaic. This does not indicate, however, any relationship between these two diseases. Curley top on bean usually causes a very marked stunting of the plants and very frequently, if infection occurs early, the plant is killed outright. This never occurs with plants affected with rugose mosaic. The presence of a Rickettsia-like organism in the affected plants may have no significance whatever, but it may account for the ruffling and savoying that characterize this disease. With *Rickettsiae* so commonly present in many kinds of insects, including the sucking insects, it would be surprising if these organisms were not found associated with virus diseases. The role of these organisms in sugar beet plants affected with curly top has not been determined and further work is necessary with the organism cultured from bean plants affected with rugose mosaic before

any conclusions can be drawn. It is necessary, first of all, to work out some method of growing the organism in subplants. Until this is done, inoculation experiments cannot be carried out with any degree of satisfaction.

CYTOLOGICAL OBSERVATIONS

The original material for the cytological studies was collected from the planting of beans in which the disease was first observed in 1927. According to Mr. George E. Starr, who had grown this bean through eight previous generations, there had been no evidence of this disease in the earlier plantings. Infection of the plants from which the seed was obtained for the 1927 planting must have occurred during the growing season of 1926. It does not seem at all probable that plants presenting such an unusual and conspicuous appearance previously could have been overlooked. Slides were prepared from the leaf material during the winter of 1927-28, but they were not studied until after the isolations were made from diseased plants in April, 1928. After the pleomorphic organism had been cultured from diseased plants grown in the greenhouse, a study was made of the slides stained from iron haematoxylin. In some of the leaf sections, the chloroplasts were found to be heavily infected with an organism indistinguishable from the one that had been cultured from the young seeds of rugose mosaic plants. All of the forms of the organism that had developed in the cultures were observed in the affected chloroplasts.

Infection of the chloroplasts by visible organisms has been observed only in localized areas of the leaf, more commonly in the vicinity of the vascular bundles. The palisade and mesophyll cells are hardly distinguishable in the regions where infection has occurred (Plate X, B) and there appears to be some hyperplasia of the mesophyll tissue where the chloroplasts are most heavily infected. In some of the sections, all of the cells near the leaf traces are heavily infected while those a short distance away contain chloroplasts that appear to be entirely normal. The infected chloroplasts contain few if any starch grains while the normal ones usually are filled with them.

Many of the infected chloroplasts show no evidence of disintegration, but this frequently has been observed in the chloroplasts of the palisade and mesophyll cells. The staining reactions of infected chloroplasts are different from those of normal ones. The haematoxylin stain is retained in the normal chloroplasts much longer than in the infected ones, and for this reason it is difficult to stop the destaining at just the right place in order to prevent the extraction of the color from the organisms within the chloroplast. In the chloroplasts undergoing dissolution, none of the large forms of the pleomorphic organism have been observed, but in others, especially in the later stages, small coccus or rod-like bodies resembling *Rickettsiae* have been observed faintly stained with the haematoxylin (Plate XI, A-B). Complete disintegration of the chloroplast may take place without any visible evidence that organisms are present.

All of the various forms of the organism that developed in the cultures have been observed in the sections of the leaf material collected in 1927. They were not found in the 1928 material, although it was from these plants that the organism was isolated. The failure to find the organism in sections from the leaves of plants in which they must have been present may have been due to a localization of the infected areas and the failure to obtain sections from those regions. This may account for the difficulty in demonstrating the organism in sections of leaves of diseased plants, or the organism

may be present in a form not readily seen in the stained sections. However, it might be contended that the failure to observe the organism in some of the plants that have been studied is evidence that it does not occur with any regularity in diseased plants and that it is probably only an occasional secondary invader. It must be admitted that the isolation of the pleomorphic organism from only a small number of the plants that have been studied is insufficient evidence on which to base any conclusions as to the probable relation of the organism to the disease with which it has been found associated. However, the extreme pleomorphism which has characterized the development stages of the organism in culture and the difficulty of cultivating it in ordinary media are characteristics which make detection in the plant and isolation in culture very difficult. The isolation from many diseased plants of coccoid and Rickettsia-like organisms, forms which developed in the cultures of the organism isolated in 1928, suggests a connection of these organisms with the one observed in the chloroplasts.

The infected chloroplasts in many cases are filled with the bacteria and the same extreme polymorphism which characterized the organism in the cultures is seen in the various forms that occur in these organs. (Plate X, C-E). Some of the polymorphic forms include slightly curved rods, curved rods swollen at only one or at both ends, v-shaped and branched rods with one or more of the tips of the branches swollen into a vesicle, swollen rods containing coccoid or diplococcoid bodies, small coccus, and Rickettsial forms. Normal-appearing chloroplasts frequently may be seen adjacent to those containing one or more organisms. There is a gradual decrease in the number of infected chloroplasts as the distance from the infection center increases. In most cases, infection of the chloroplasts apparently began in the cells bordering the vascular bundles. Since the organism is transmitted through the seed, it seems very probable that the portal of entry to the ovules must be the vascular tissues. The occurrence of heavily infected chloroplasts in the cells immediately adjacent to the vascular bundles lends weight to the probability of vascular movement of the organism.

Sections were made from the leaf tissues of 10 typically diseased plants during the summer of 1930. The organism, as it was seen in the 1927 material, was not observed in any of the sections. Tissue changes were seen in some of the material that were also observed in the previous studies. Chloroplast disintegration of the mesophyll and palisade cells was observed and in some of the sections this disintegration had resulted in the complete destruction of the plastids. In the disintegrating chloroplasts of the mesophyll parenchyma cells, extremely small bacterium-like bodies were observed in large numbers. These were seen only in sections where the destaining had removed all of the dye from the gelatinous mass of the chloroplast detritus and had left enough color in the included bodies to make them visible (Plate XI, A-B). These bodies have been found in sections from many of the plants but their exact nature has not been determined. Masses of these bodies may be observed distributed irregularly throughout the cells in which the chloroplasts have been destroyed. They stain but faintly with haematoxylin but retain the stain longer than the disintegrating ground tissue of the diseased chloroplast.

The cytological studies have shown that a bacterial organism similar in all respects to the organism isolated in 1928 from the young seeds of Refugee plants affected with rugose mosaic occurs in the chloroplasts of plants affected with this disease. The isolation of the organism from plants two generations removed from those in which it was first observed indicates that it may be more than a transient invader. Even though the organism was not

demonstrated by cytological methods in the plants grown in 1928, it was obtained in cultures from the young seeds of these plants. These facts make cytological observations of uncertain value in determining the presence of the organism in diseased plants. Cocci and Rickettsia-like organisms have been isolated from young seeds of diseased plants, but the cocci have not shown any marked polymorphic tendencies in the cultures, and the Rickettsial forms have not been subcultured. The working out of a successful method of growing them in subplants is one of the problems requiring solution before they can be studied extensively.

PATHOGENESIS

Subcultures of the cocci isolated from navy pea beans have been inoculated by several methods into the young leaves of healthy bean plants of several susceptible varieties. The methods tried have included hypodermic injections, leaf mutilation, and the pricking of the inoculum into the leaf tissues with fine needles. In some of the experiments, infection of the inoculated plants appeared to have occurred after an incubation period comparable to that required for the disease to develop following inoculation with infectious juice, but the results of these tests have not been confirmed by subsequent inoculation experiments. Local lesions have been produced with some of the cultures but a summation of the results of the inoculation experiments indicates that the cocci as they occur in the cultures made from diseased plants can not, when inoculated into susceptible varieties of beans by the methods that have been tested, produce a diseased condition comparable to mosaic.

Due to difficulties encountered in subculturing the pleomorphic organism isolated from Refugee bean plants affected with rugose mosaic only one inoculation test was attempted. Ten young Refugee seedlings were inoculated with one of the tissue cultures of the organism by means of hypodermic injections into the leaf veins and by leaflet mutilation. A marked bronzing of the veins of some of the inoculated leaflets was evident in five days after the inoculations were made but none of the plants developed typical symptoms of rugose mosaic.

DISCUSSION

In beginning a discussion of the results that have been presented in the preceding sections, the need is recognized for a more complete investigation of some of the questions that have arisen in connection with certain portions of this study. Some inconsistencies which have arisen in the studies of the bacteriology of field-grown plants apparently free from mosaic have not been completely harmonized with the general results of the bacteriological studies and further investigation is necessary with plants grown under a wider range of conditions. The results of the bacteriological studies may, therefore, be considered as of a preliminary nature until more extensive tests have been completed. The apparent inconsistencies revealed, however, have been the exception rather than the rule and in working with

plants grown under closely controlled conditions these conflicting results have largely been eliminated. However, since the question of the comparative bacteriology of mosaic and normal plants is of outstanding interest, the writer feels justified in deferring a final statement until more work is completed.

The seed transmission experiments with bean mosaic have yielded evidence that relates the movement of the virus through the plant to the vascular tissues. The probability of a vascular migration is strengthened somewhat by the preliminary studies on the anatomy of the pod and the vascular features which make it possible to explain the occurrence of infected and non-infected seeds within the same pod. There is some evidence from the investigations of other virus diseases that the spread of the causal agents through plant tissue can best be explained by associating their movement with these tissues. The work of Bennett (1927) with raspberry curl and of Holmes (1930) with tobacco mosaic cannot be reconciled with the assumption that these viruses move solely through the ground tissues of the plant. It may not be improbable that there is some movement through these tissues, particularly in the meristematic regions where vascular elements are as yet undifferentiated. Following the inoculation of some viruses into various meristematic regions, rapid multiplication apparently takes place followed by an immediate and extremely rapid migration through the plant. In curly top of sugar beets, Severin (1924) and in maize streak, Story (1928) have demonstrated a rate of distribution that cannot be explained by diffusion mechanics or other methods that are associated with the movement of water or elaborated nutrient materials. Nothing is known concerning the mechanics of this distribution, but some power of autonomous movement independent of diffusion must be presupposed, both because of the rate of movement and of the particulate nature of the viruses which would preclude diffusion as a distributor of the virus particles. Little is known about the rate of movement of the viruses belonging strictly to the mosaic group. McCubbin and Smith (1930) have determined the rate of movement of the tomato mosaic virus at 1 to 2 cm. per hour. By ringing experiments, they showed also that the virus was not prevented from migrating through the regions in which sections of the woody cylinder and of the cortex had been removed below the points of inoculation. These experiments add weight to the probability of vascular distribution of the viruses, since in tomato and other solanaceous plants there is present an inner phloem that would allow an uninterrupted passage of the virus.

The experiments on seed transmission of bean mosaic, together with the preliminary studies on the vascular anatomy of the pod, suggest that a plausible explanation for the irregular transmission of the virus to the seeds is to be found in assuming that the virus is present in only a part of the vascular elements which supply the developing ovules. Since there is no direct vascular connection between the mother plant and the embryo, which is nourished entirely by the diffusion of soluble materials, the question arises as to how the virus reaches the cells of the embryo. The vascular strands penetrate the inner integument (Plate VI, D), but there are no visible conducting elements that extend farther into the ovule. How then can the virus bridge the network of cells that separate the embryo sac and the vascular strands in the integument? This question cannot be answered with certainty at the present time, but one suggestion may be offered that seems to be in accord with a well authenticated observation in connection with seed transmission of the virus. It has been pointed out by Fajardo (1930) that

seed transmission of bean mosaic rarely occurs in plants that become infected after flowering. Disregarding for the present the possibility of genetical factors being concerned, this fact suggests that it is necessary for the virus to reach the ovule before or shortly after fertilization occurs. When the ovule is young, and up to the time that fertilization occurs, it is composed of embryonic cells with uninterrupted cytoplasmic connections extending from cell to cell. The embryo sac is nourished by food that diffuses through the chalazal end. After fertilization, the nucellus is absorbed by the developing cells of the embryo sac. While the cells of the ovule are yet in the meristematic condition, it would be theoretically possible for the virus, after being carried into the ovary through the vascular strands of the funiculus, to pass from cell to cell through the plasmodesma until it reached the cells of the embryo sac. If the mother plant were infected early enough so that the virus could reach the ovule before the meristematic phase of development had been passed, it would be able to infect some cells of the embryo and be transmitted to the tissues of the sporophyte. If infection occurred after flowering, the virus might not be able to reach the ovule until after the meristematic tissues adjacent to the point of termination of the vascular strands had undergone differentiation into parenchyma tissue, the cells of which might form an impassable barrier. The presumption that the virus cannot pass through parenchyma cells which have reached complete development has some measure of support in the fact that inoculations of the virus into non-meristematic tissues usually fails to produce infection. That other factors may be concerned in the inability of the viruses to set up infections in mature tissues is a possibility that must be recognized. The hypothesis just given to explain the variability of seed infection is one, however, that can be reconciled reasonably well with the known facts of infection phenomena in bean mosaic.

In the cytological studies, it has not been possible to demonstrate bacteria consistently in the chloroplasts of the leaf and petiole parenchyma cells of mosaic bean plants. This may seem a very strong argument against an assumption that these organisms have any causal relation to the disease. It might reasonably be concluded from the inoculation experiments that the organisms represent occasional secondary invaders that gain ingress through wounds and find in the sick plants favorable conditions for multiplication. Against this conclusion, there cannot be directed at present any experimental evidence. It has been fairly well established by the isolation experiments that the cocci can be cultured readily from mosaic plants by the methods that have been described and that their occurrence in the young seeds in many cases parallels the percentage transmission of the virus in the seeds harvested from mosaic plants. This may be a mere coincidence, but the seemingly close association of virus and coccus does not support this explanation. The failure to demonstrate the organism regularly in the diseased chloroplasts can not, in view of the multiplicity of forms which are known to occur in the ontogenetic development of the bacteria, be construed as conclusive evidence that it is not present in some other form. The pleomorphism which characterized the development of the organism isolated in 1928 from plants affected with rugose mosaic is suggestive of what may occur in diseased plants and is, in fact, what was shown to occur in the chloroplasts of plants affected with this disease. The polymorphism and failure to make any initial growth in any medium except the bean pod juice, in some instance requiring for continued existence the presence of tissue, are characteristics of the organisms isolated from the bean plants which would seem to relate them

to parasitic forms. To conclude, however, that they represent the causal agents of the diseases with which they are associated would be an unwarranted interpretation of the evidence.

It is evident that the bacteriological studies are incomplete and that much is omitted which may seem essential. Studies of the cultural reactions which are necessary for the identification of the organisms have been deferred until such time as the need for this information seems more essential than at present. Some preliminary tests have been conducted with a limited number of cultures isolated from mosaic navy pea bean plants. It has been pointed out earlier that there is a great variation in the size and morphology of this organism. Usually, it is obtained in the original cultures from the young seeds as a typical *Micrococcus* occurring singly or in pairs, but in some cases the *Streptococcus* form appears in the original culture. In nearly every case, subcultures are found to contain the typical single or paired forms. Where the chain form persists, subculturing has only been possible with the aid of tissue added to the bean pod juice. The organism is gram positive and an active nitrate reducer.

Since only a limited number of cultures have been used in the preliminary tests, it can not be stated with certainty whether or not more than one species of coccus is associated with this disease. The question of the specificity of the organisms for a particular type of mosaic disease must be held in abeyance until more extensive tests have been conducted with a large number of cultures. The results that have been presented have merely attempted to demonstrate the association of cocci with mosaic disease in the bean, an association that may be of some significance as indicated by the presence of the organisms in disintegrating chloroplasts which are a constant feature of the disease. Aside from the cytological observations, however, on the presence of these organisms in the dissolving chloroplasts no evidence of their pathogenic nature has been obtained.

Since in virus disease we are dealing with causal agents that are particulate but which are apparently extremely small, how could bacteria such as have been cultivated from mosaic bean plants have any possible etiological significance? This naturally raises the question impossible to answer at the present time; that is, are the viruses stabilized monomorphic bodies or do they exist in more than one form? There is little evidence upon which to base a conclusion. Work on filtration has furnished evidence that the virus particles are not uniform in size and in the diseases where the insect vectors act apparently as alternate hosts, it is likely that the viruses are polymorphic. If the virus of a disease like tobacco mosaic is stabilized and exists in only one form, it may not be possible to cultivate it by present methods outside of the plant. If, however, the virus is potentially capable of passing into another stage of development, it might be possible by suitable methods to cultivate it in an artificial medium. Methods have been described by several investigators that are successful in forcing dissociative reactions upon bacterial cells. It is possible to begin with an organism, which, according to the Koch standards would be considered as morphologically stable, and by special cultural methods to force it into a filterable condition, a condition in which it may become stabilized or out of which it may be brought back to the original form. Should the viruses represent organisms that have originated by dissociation or otherwise from the bacteria, it might be possible by the use of the methods that have been successful in initiating the reverse reaction to force them into another stage in which they may be less refractory to cultivation outside of plant cells. This is one of the

hopeful methods of attacking the problem that has not yet been tested in the study of the plant viruses.

In conclusion, it may not be amiss to refer to some observations which indicate that some of the objections to the bacterial theory of the nature of the viruses are rapidly being overcome. The resistance to chemicals, ultra violet light and alcohol have frequently been cited as serious obstacles to the acceptance of the theory that bacteria are concerned in the causation of virus diseases. These objections are gradually being met by a study of the viruses in a purified condition. When freed from the protecting influence of coagulable materials in the expressed juice from mosaic plants, it has been shown that the suspended virus exhibits a reaction to chemicals and ultra violet light that is not unlike that of many known kinds of bacteria. These studies have been conducted with both plant and animal viruses. Olitsky (1928) has tested the resistance of the foot and mouth disease virus to chemicals and found that it reacts similarly to *Staphylococcus aureus.*

The reaction of the virus of vesicular stomatitis to ultra violet light was determined by Olitsky and Gates (1927), and they conclude: "If absorption of specific energies is an index of chemical character, the parallel reactions are indirect but suggestive that the substance of the virus is similar in character and chemical composition to bacterial protoplasm."

Experiments with the purified virus of tobacco mosaic have yielded similar results. The results from the studies that have been conducted on the viruses themselves indicate that bacteriological investigations which utilize the newer methods that have been developed in the study of dissociation hold the most hopeful outlook for the future advance in our knowledge of the nature of the viruses.

SUMMARY

1. Bean mosaic was recognized by Iwanowski in Russia as early as 1899 and in Connecticut by Clinton in 1908. It is now world wide in its distribution and has been observed in young plants grown from seed obtained from most of the important countries of the world.

2. The disease is of great economic importance in most of the leading commercial varieties of field and canning beans. Robust, a small white pea bean, is highly resistant and has largely replaced susceptible varieties of small white beans in Michigan, New York, and other sections to which it is adapted.

3. Great variation in symptoms has been noted on the same variety. The symptoms of the diseases are described in detail as they occur in the navy pea and Refugee varieties, beans highly susceptible to the disease.

4. The manner in which the symptoms develop in some plants grown from infected seed indicates that the causal agent is perhaps more or less localized in its distribution and movement through the plant. The virus apparently moves through the vascular tissues, presumably the phloem, but its exact path has not yet been determined.

5. Insect transmission of bean mosaic under controlled conditions was first accomplished in 1922, using the potato aphis *Macrosiphum solanifolii* Ashm. Field observations for several years indicate that this insect is probably not responsible for the rapid spread of the disease

that occurs in seasons favorable for its development. The bean leafhopper is present in large numbers in seasons when mosaic spreads rapidly but transmission has not yet been accomplished with this insect.

6. Bean mosaic is not a highly infectious disease like tobacco mosaic and is, therefore, transmitted artificially with much greater difficulty. Leaf mutilation and other methods successful in transmitting mosaic in other plants usually fail with this disease. The difficulty in transmitting the disease may be due, in part, to the unequal distribution of the virus in the plant.

7. The virus is transmitted through the seed in variable percentages. In plants grown from infected seed, the virus is transmitted to approximately one-half of the seeds. Details are given of an experiment in which seeds from pods harvested from 50 primary-infected mosaic plants were indexed for mosaic infection. The position of the seed in the pod does not determine whether or not it will receive the virus, the chances are about equal that seeds in any position may escape infection.

8. Plants infected during the growing season transmit mosaic to the seed in a much lower percentage than those that are infected throughout the growing season. In most cases, infections that occur after flowering do not reach the seeds.

9. Evidence is presented to show that a plausible explanation for the variability of mosaic transmission in the seed may be found in the vascular anatomy that characterizes the bean pod. The type of vascular anatomy and the apparent occurrence of localized infections in some plants may account for the occurrence of infected and non-infected seeds within the same pod.

10. With the exception of Robust, all of the important commercial varieties of field and canning beans are susceptible to mosaic. The reaction to mosaic of beans belonging to other genera is given.

11. Observations in 1927 of sections from the petioles of mosaic bean plants indicated that organisms were present in the disintegrating tissues of the chloroplasts of the cortical parenchyma cells. Studies of freehand sections of living tissue have shown that the areas in which the organisms occur are localized and sometimes difficult to find. In some plants, they may not be found at all. The organisms are present in the lytic areas of the chloroplasts in the cortical parenchyma of the petioles and also in the leaf parenchyma cells.

12. The normal chloroplast and its inclusions have been studied by the same method and no organisms similar to those found in the mosaic plants have been detected. Chloroplast disintegration may occur with no evidence of the organism being present. The organism occurs in the affected cells as single or paired cocci. Extremely small, round, or coccoid bodies less than 0.5 microns in diameter have sometimes been observed in enormous numbers filling the chloroplasts in the leaf parenchyma cells of mosaic plants. They have not been observed elsewhere nor in the chloroplasts from normal plants.

13. Bodies similar in size and shape to those observed in the chloroplasts have been found in the border parenchyma cells of the phloem and xylem. They have been demonstrated in material treated with chrom-osmic-acetic killing and fixing agent and are believed to represent the organisms observed in the chloroplasts. Mitochondria may be

confused with these bodies in fresh material or in sections treated with mitochondria-preserving killing and fixing solutions.

14. Using a medium consisting of unheated and undiluted juice from the young pods of mosaic-susceptible beans, an organism similar morphologically to the coccoid bodies observed in the diseased chloroplasts has been isolated repeatedly both from the tissues of mosaic plants grown under sterile conditions in large test tubes and from the young seeds removed with aseptic precautions from pods produced on mosaic plants. From field-grown susceptible varieties, like the Refugee and Black Valentine, a similar organism has been isolated in a small percentage of cases from the young seeds of normal-appearing plants.

15. The number of infected seeds in pods from mosaic plants varies widely and there is a close correlation between the percentages of infected seeds on mosaic plants and the number of seeds infected with the mosaic virus. In plants infected during the growing season and previous to flowering, the number of seeds infected with the organism is reduced in the same proportion as the number of seeds containing the virus is fewer than those from plants grown from infected seed.

16. Rugose mosaic, found so far only on the Refugee variety, appears to be distinct from the true mosaic to which the Refugee varieties are extremely susceptible. Diseased field-grown plants show an extreme ruffling and savoying of the leaflets with no evidence of mottling. In the greenhouse the rugosity of the leaflets is less intense and the plants may be more or less chlorotic with some mottling along the veins.

17. An extremely pleomorphic organism has been demonstrated in the chloroplasts of leaflets from Refugee plants affected with rugose mosaic in 1927. The areas of infected leaf tissue were localized and occurred mostly in the regions of the vascular bundles. Organisms with a similar morphology were not found in the 1930 material.

18. Extremely small bodies, resembling minute cocci, have frequently been observed in the living chloroplasts of leaflets affected with rugose mosaic. These bodies seem to be similar to those seen in the chloroplasts from plants affected with the true mosaic disease.

19. The pleomorphic organism demonstrated in the chloroplasts has been isolated from young seeds of Refugee plants affected with rugose mosaic. The organism was isolated from seeds two generations removed from the original material in which it was first observed.

20. The organism was cultured only in the bean pod extract containing living tissue and the growth in this medium produced but a slight clouding of the liquid.

21. All of the many forms of the organism observed in the affected chloroplasts were produced progressively in the subplants. With the occurrence of an extremely small coccus stage, the organism could no longer be induced to grow in any of the media tested.

22. Cocci and bacterium-like organisms resembling the *Rickettsiae* have also been cultured from plants affected with rugose mosaic. The Rickettsia-like organisms have not yet been grown in subplants, and they have not been isolated from diseased plants with the same frequency as the cocci which can be subcultured in the bean pod extract.

23. Inoculations of the cocci isolated from plants affected with true mosaic disease and of the pleomorphic organism isolated from plants affected with rugose mosaic have not resulted in the reproduction of mosaic disease in the inoculated plants.

ACKNOWLEDGEMENTS

The writer is greatly indebted to Dr. C. H. Kauffman of the University of Michigan and to Dr. E. A. Bessey of Michigan State College for unfailing encouragement and helpful advice during the course of this investigation. Dr. Bessey also aided with the manuscript. Dr. Philip Hadley has supplied many helpful suggestions and much encouragement in the bacteriological studies.

BIBLIOGRAPHY

Anonymous. Periods for which seeds retain their germinating power. Cyprus Agr. Jour. *15:* 213. 1920.

Archibald, E. S. Bean mosaic. Canada Dept. Agr. Exp. Farms Rept. Botanist. *1919-1920. 62.* 1921.

Barss, H. P. Bean mosaic. Oregon Agr. Exp. Sta. Third Crop Pest and Hort. Rept. 1915-1920: 192-196. 1921.

Beijerinck, M. W. Ueber ein Contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabakblätter. Centralb. Bakt. 2 Abt. *5:* 27-33. 1899.

Bennett, C. W. Virus diseases of raspberries. Mich. Agr. Exp. Sta. Tech. Bul. 80. 1927.

Bewley, W. F. Minute "organisms" isolated from the virus of mosaic disease of tomato. Nature *112:* 903. London. 1923.

———— and W. Corbett. The control of cucumber and tomato mosaic disease in glass houses by the use of clean seed. Ann. App. Biol. *17:* 260-266. 1930.

Blodgett, F. M. and Karl Fernow. Testing seed potatoes for mosaic and leaf roll. (Abst.) Phytopath. *11:* 58-59. 1921.

Boncquet, P. A. Presence of nitrites and ammonia in diseased plants. Jour. Am. Chem. Soc. *38:* 2572-2576, 1916.

Brandenburg, E. Ueber Mosaikkrankheiten an Compositen. Forsch. Gebiet Pflanzenkr. u. Immunität Pflanzenreich *5:* 39-72. 1929.

Brewer, P. H., James B. Kendrick, and Max W. Gardner. Effect of mosaic on carbohydrate and nitrogen content of the tomato plant. Phytopath. *16:* 843-851. 1926.

Burkholder, W. H. Bean diseases in New York State in 1916. Phytopath. *7:* 61. 1916.

———— and A. S. Muller. Hereditary abnormalities resembling certain infectious diseases in beans. Phytopath. *16:* 731-737. 1926.

Carsner, Eubanks. Attenuation of the virus of sugar beet curly top. Phytopath. *15:* 745-757. 1925.

———— Susceptibility of the bean to the virus of sugar beet curly top. Jour. Agr. Res. *33:* 345-348. 1926.

———— and C. F. Stahl. Studies on curly top disease of the sugar beet. Jour. Agr. Res. *28:* 297-319. 1924.

Clinton, G. P. Conn. Agr. Exp. Sta., Rept. of Botanist. *1908:* 859-860. 1909.

Cook, M. T. The effect of some mosaic diseases on cell structure and on the chloroplasts. Jour. Dept. Agr. Porto Rico *14:* 69:101. 1930.

Cowdry, E. V. The distribution of Rickettsia in the tissues of insects and arachnids. Jour. Exp. Med. *37:* 431-457. 1923.

Dickson, B. T. Studies concerning mosaic diseases. Macdonald College Tech. Bul. 2. 1922.

Ducomet, Vital. Observations et expériences sur les maladies dégénérescence de la pomme de terre. Bul. Soc. Path. Veg. France. *9:* 29-38. 1922.

Duggar, B. M. The problem of seed transmission of the typical mosaic of tobacco. (Abst.) Phytopath. *20:* 133. 1930.

Eckerson, Sophia H. An organism of tomato mosaic. Bot. Gaz. *81:* 204-208. 1926.

Elmer, O. H. Transmissibility and pathological effects of the mosaic disease. Iowa Agr. Exp. Sta. Res. Bul. 82. 1925.

Fajardo, T. J. Studies on the mosaic disease of the bean (*Phaseolus vulgaris* L.) Phytopath. *20:* 469-494. 1930.

———— Studies on the properties of the bean mosaic virus. Phytopath. *20:* 883-888. 1930.

Feigin, Bronislawa. On the nature of the etiologic agent of typhus fever. Jour. Prev. Med. *3:* 391-404. 1929.

Fernow, K. H. Interspecific transmission of mosaic diseases of plants. Cornell Agr. Exp. Sta. Memoir 96. 1925.

Gardner, Max W. and James B. Kendrick. Potato leaf roll in Indiana. Purdue Univ. Agr. Exp. Sta. Bul. 284. 1924.

Gloyer, W. O. Two new varieties of red kidney bean: Geneva and York. New York State Agr. Exp. Sta. Tech. Bul. 145. 1928.

Goldstein, Bessie. A cytological study of the leaves and growing points of healthy and mosaic diseased tobacco plants. Bul. Torr. Bot. Club *53:* 499-599. 1926.

Goldsworthy, M. C. Attempts to cultivate the tobacco mosaic virus. Phytopath. *16:* 873-875. 1926.

Grainger, J. An attempt to cultivate the virus of tobacco mosaic in vitro. Proc. Leeds Phil. and Lit. Soc. Sci. *2:* 33-35. 1929.

————. The appearance of bean mosaic in England. Proc. Leeds Phil. and Lit. Soc. Sci. *2:* 32. 1929.

Güssow, H. T. Bean mosaic. Canada Dept. Agr. Exp. Farms. Interim Rept. of Botanist. *1921-1922:* 26-27. 1922.

Hadley, Philip, Edna Delves and John Klimek. The filtrable forms of bacteria; 1. A filtrable stage in the life history of the Shiga dysentery bacillus. Jour. Infect. Dis. *48:* 1-159. 1931.

Harter, L. L. Bean diseases in the West. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rpt. *11:* 148-149. 1927.

Hauduroy, P. Technique de culture des formes filtrantes invisibles des microbes visibles. Compt. rend. Soc. biol. *97:* 1392. 1927.

———— Les ultravirus et les formes filtrantes des microbes. Paris, Masson & Cie. 1929.

Hoggan, Isme A. Further studies on aphid transmission of plant viruses. Phytopath. *21:* 199-212. 1931.

Holmes, Francis O. Local and systemic increase of tobacco mosaic virus. Amer. Jour. Bot. *17:* 789-805. 1930.

Horsfall, J. G. Diseases of canning crops. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. Sup. *76:* 83-89. 1930. (Mimeographed.)

Iwanowski, D. Ueber die Mosaikkrankheit der Tabakspflanze (lu le 12 février 1892). Bul. de l'Académ. Imper. d. Sciences d. S. Petersbourg, Nouvelle Série *3:* 35: 67-70. 1894.

———— Ueber die Mosaikkrankheit der Tabakspflanze. Central. Bakt. Abt. *2,* 5: 250-254. 1899.

———— Ueber die Mosaikkrankheit der Tabakspflanze. Zeitschr. Pflanzenkr. *13:* 1-41. 1903.

Johnson, James. The attenuation of plant viruses and the inactivating influence of oxygen. Science (N. S.) *64:* 210. 1926.

———— The classification of plant viruses. Wis. Agr. Exp. Sta. Res. Bul. 76. 1927.

Kasai, M. Observations and experiments on the leaf roll disease of the Irish potato in Japan. Ber. Ohara Inst. für landw. Forsch. *2:* 47-77. 1921.

Kendrick, J. B. and Max Gardner. Soy bean mosaic: Seed transmission and effect on yield. Jour. Agr. Res. *27:* 91-98. 1924.

Klebahn, H. Experimentelle und Cytologische Untersuchungen im Anschluss an Alloiophyllie und Viruskrankheiten. Planta. Archiv für Wiss. Botanik *6:* 40-95. 1928.

Kunkel, L. O. Studies on aster yellows. Amer. Jour. Bot. *13:* 646-705. 1926.

Leach, J. G. The effect of grafting on resistance and susceptibility of beans to *Colletotrichum lindemuthianum.* Phytopath. *19:* 875-877. 1929.

Loeb, J. Proteins and the theory of colloidal behavior. Edition 2. 380 p. 1924.

McClintock, J. A. Lima bean mosaic. (Abst.) Phytopath. *7:* 60-61. 1917.

McCubbin, W. A. and Floyd F. Smith. Spread of Mosaic virus in tomato plants. (Abst.) Phytopath. *20:* 134. 1930.

Mayer, A. Ueber die Mosaikkrankheit des Tabaks. Landw. Versuchs Sta. *32:* 450-467. 1886.

Melhus, I. Mosaic studies. (Abst.) Phytopath. *12:* 42. 1922.

Merkel, Ludwig. Beiträge zur Kenntnis der Mosaikkrankheit der Familie der Papilionaceen. Zeitschr. Pflanzenkr. *39:* 289-347. 1929.

Mulvania, M. Cultivation of the virus of tobacco mosaic by the method of Olitsky. Science *62:* 37. 1925.

Nelson, R. Transference of the bean mosaic virus by *Macrosiphum solanifolii.* Science N. S. *56:* 342-344. 1922.

———— The occurrence of protozoa in plants affected with mosaic and related diseases. Mich. Agr. Exp. Sta. Tech. Bul. 58. 1922.

Ogilvie, L. Preliminary report of the Plant Pathologist. Bermuda Board of Dept. Agr. Rept. *1923:* 28-34. 1924.

Olitsky, Peter K. Experiments on the cultivation of the active agent of mosaic disease of tobacco and tomato. Science *60:* 593-594. 1924.

———————— Experiments on the cultivation of the active agent of mosaic disease in tobacco and tomato plants. Jour. Exp. Med. *41:* 129-136. 1925.

———————— and F. L. Gates. The reaction of vesicular stomatitis virus and ultra violet light. Proc. Soc. Exp. Biol. and Med. *24:* 431-432. 1927.

————————, J. Traum and H. W. Schoening. Report of the foot and mouth disease commission of the U. S. Department of Agriculture. U. S. Dept. of Agr. Tech. Bul. *76.* 1928.

Pierce, W. H. and C. W. Hungerford. Symptomatology, transmission, infection and control of bean mosaic in Idaho. Idaho Agr. Exp. Sta. Res. Bul. *7.* 1929.

Porter, R. H. A preliminary report of surveys for plant diseases in East China. (U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. Sup. *46.* 1926.)

Purdy, Helen A. Attempt to cultivate an organism from tomato mosaic. Bot. Gaz. *81:* 210-217. 1926.

Quanjer, H. M. The mosaic disease of the Solanaceae, its relation to the phloem-necrosis, and its effect upon potato culture. Phytopath. *10:* 35-47. 1920.

Randolph, L. F. Cytology of chlorophyll types of maize. Bot. Gaz. *73:* 337-375. 1922.

Rands, R. D. and Wilbur Brotherton, Jr. Bean varietal tests for disease resistance. Jour. Agr. Res. *31:* 101-154. 1925.

Reddick, D. and V. B. Stewart. Varieties of beans susceptible to mosaic. Phytopath. *8:* 530-534. 1918.

———————— Additional varieties of beans susceptible to mosaic. Phytopath. *9:* 149-152. 1919.

Schaffnit, E. and H. Weber. Ueber das Vorkommen von intrazellularen Körpern in den Geweben mosaikkranker Rüben. Forsch. Gebiet Pflanzenkr. u. Immunität Pflanzenreich *4:* 23-42. 1927.

Severin, H. H. P. Curly leaf transmission experiments. Phytopath. *11.* 80-93. 1924.

Sheffield, F. M. L. and J. Henderson Smith. Intracellular bodies in plant virus diseases. Nature (London) *125:* 200. 1930.

Smith, Fanny Fern. Some cytological and physiological studies of mosaic diseases and leaf variegations. Ann. Mo. Bot. Gard. *13:* 425-484. 1926.

Smith, J. Henderson. Experiments with a mosaic disease of tomato. Ann. App. Biol. *15:* 155-167. 1928.

Smith, Ralph E. and P. A. Boncquet. Connection of a bacterial organism with curly top of the sugar beet. Phytopath. *5:* 335-341. 1915.

Sorokin, Helen. Phenomena associated with the destruction of the chloroplasts in tomato mosaic. Phytopath. *17:* 363-379. 1927.

Spragg, F. A. and E. E. Down. The Robust Bean. Mich. Agr. Exp. Sta. Special Bul. 108. 1921.

Stewart, V. B. and Donald Reddick. Bean mosaic. (Abst.) Phytopath. *7:* 61. 1917.

Stone, R. E. and J. E. Howitt. Plant diseases and fungi comparatively new or rare in Ontario. Phytopath. *10:* 317. 1920.

Storey, H. H. Transmission studies with maize streak disease. Ann. App. Biol. *15:* 1-25. 1928.

Swezy, Olive and Henry H. P. Severin. A Rickettsia-like micro-organism in Eutettix tenellus (Baker), the carrier of curly top of sugar beets. Phytopath. *20:* 169-178. 1930.

United States Department of Agriculture. Bureau of Plant Industry. Diseases of Bean. U. S. Dept. of Agr., Bur. Plant Indus. Plant Dis. Bul. Sup. *10:* 226-232. 1920.

Wright, W. H. and Vladimir Skoric. The demonstration of bacteria in plant tissues by means of the Giemsa stain. Phytopath. *18:* 803-807. 1928.

Zaumeyer, W. J. Bean diseases in western United States in 1929. U. S Dept. Agr., Bur. Plant Indus. Plant Dis. Rpt. *14:* 38-43. 1930.

Zirkle, C. The structure of the chloroplast in certain higher plants. Amer. Jour. Bot. *13:* 301-340. 1926.

EXPLANATION OF PLATES

Plate I. A Navy pea bean plant affected with mosaic. One of the simple leaves and the 3 leaflets of the first compound leaf show the mottling that is typical of the true mosaic disease.

B Variation in mosaic symptoms shown by the first compound leaf of the Refugee variety. Plants grown from infected seed. 1 mottling, 2 curling, 3 chlorosis along veins, 4 malformations of leaflets, 5 slight rugosity of the lamina and clearing of the veins. Photographed by transmitted light.

Plate II. A A field-grown plant of the navy pea variety showing excessive branching, severe curling and blistering of the leaflets due to mosaic infection.

B Pseudo-mosaic, the effect of high temperature. A rugose appearance accompanied by severe curling of the leaflets occurs in some varieties of beans during periods of high temperature in July and August. Photographed in July 1919.

Plate III. A Multiflory in a navy pea bean plant affected with mosaic. No wilting of the leaves was apparent until after the plant was transplanted to the pot for photographing. September 1920.

B Method of growing bean plants under sterile conditions in large test tubes.

C A mosaic navy pea bean plant grown under sterile conditions for use in the bacteriological studies.

Plate IV. A Three mosaic-free Refugee bean seedlings.

B Refugee bean plants affected with rugose mosaic showing the mild rugosity of the laminae on greenhouse grown plants.

Plate V. A Severe symptoms of rugose mosaic on a field-grown Refugee bean plant. The plant is dark green in color and free from mottling in the leaflets.

B Two pods from a Refugee bean plant affected with rugose mosaic. Frequently the rugose symptoms which characterize the effect of the disease on the leaves are also observed on the pods.

Plate VI. A Primary compound leaf from a mosaic Refugee bean plant grown from infected seed. The chlorosis which is developing in the tissues adjacent to the midribs is evidence for a vascular movement of the virus.

B Primary compound leaf from a mosaic navy pea bean plant grown from infected seed. Evidence of a vascular migration of the virus is seen in the progressive

development of hyperplastic dark green areas along the midrib and lateral veins.

C-D Sections through adjacent seeds in an immature bean pod. The freshly cut sections were treated with concentrated HCl, followed by an aqueous solution of phloroglucin. The ovules are attached by their respective funiculi alternately to the 2 parallel, ventral, vascular bundles in the placental tissue at the ventral suture. x 8.

E An immature bean pod opened to show the arrangement of the seeds which are attached alternately to the 2 ventral vascular bundles.

Plate VII, A Localized effect of mosaic in the first compound leaf on a navy pea bean plant grown from infected seed. Two leaflets and a portion of the third one show the dwarfing and other effects of the disease.

B A primary compound leaf from a mosaic navy pea bean plant grown from infected seed. Typical mosaic mottling is seen in the right hand leaflet and a small sector in the lower right hand corner of the terminal leaflet is beginning to show a faint mottling. These localized effects of the disease are apparently indicative of a restricted vascular infection. Photographed by transmitted light.

C Photomicrograph of a section through the cortical parenchyma of a petiole from a navy pea bean plant affected with mosaic. The chloroplasts are filled with small, elongated, tapering bodies which in some cases show differential staining reactions. Similar chloroplast inclusions are sometimes found in normal-appearing bean plants. Iron-haematoxylin stain. x 1380.

D Freehand, unstained, tangential section of living cells from the cortical parenchyma of a petiole from a mosaic navy pea bean plant. The chloroplasts show marked distintegrative changes and are filled with small starch granules. One cell at the lower left shows normal chloroplasts oriented around the peripheral portion of the cell. Photographed with 2 mm. oil immersion objective, N. A. 1.40, 15 x periplan ocular, Wratten M plate, Wratten B screen. x 1380.

Plate VIII, A-C Freehand, tangential sections from the cortical parenchyma tissue of mosaic navy pea bean plants. Iron haematoxylin stain after chrom-osmic-acetic killing and fixing solution. The chloroplasts are in advanced stages of disintegration and contain large numbers of very small, single and paired cocci. C an early stage; B shows almost complete destruction of the chloroplasts with large numbers of the cocci in the residual material. x 1380.

D Paraffin section from the cortical parenchyma tissue of a petiole of a mosaic navy pea bean plant. Short chains of coccoid bodies are seen close to the cell wall. Chrom-osmic-acetic killing and fixing agent, iron-haematoxylin stain. x 1380.

E Tangential section through the cortical parenchyma tissue of a petiole from an apparently normal navy pea bean plant. The chloroplasts have a normal orientation and contain one or more large granules of starch. No other inclusions are visible. Chrom-osmic-acetic killing and fixing agent, iron-haematoxylin stain. x 1104.

Plate IX, A-B Tangential sections through the conducting tissues of petioles from mosaic navy pea bean plants. Small, single or paired coccoid bodies are present in the cytoplasm of the border parenchyma cells of the phloem and xylem. Chrom-osmic-acetic killing and fixing solution, iron haematoxylin stain. x 1380.

C-E Cocci cultured from young seeds of mosaic navy pea bean plants. Cover slip preparations from pure cultures. Giemsa stain. At C long chains with giant cells present; D paired or diplococcoid forms. E short chains breaking up into diplococcoid forms. x 1380.

Plate X, A-B Transverse section of a leaf from a Refugee bean plant affected with rugose mosaic. Chrom-osmic-acetic killing and fixing agent, iron-haematoxylin stain. The chloroplasts have been decolorized by the iron alum but contain deeply staining bacteria in varying numbers. The tissues show a hyperplastic development but with no differentiation into palisade and mesophyll parenchyma. A vascular bundle is seen at the lower left in B. x 792.

C-E Sectors from the sections shown in A and B photographed with 2 mm. apochromatic oil immersion objective, N. A. 1.40, periplan ocular 15 x. Magnification 1380. The extreme polymorphism of the organism is detectable in the infected chloroplasts.

Plate XI, A Transverse section of the mesophyll parenchyma tissue of a Refugee bean leaflet affected with rugose mosaic. The chloroplasts are in the final stages of disintegration and contain numerous, very small, faintly staining, rod-like or coccoid bodies. Chrom-osmic-acetic killing and fixing agent, iron-haematoxylin stain. x 792.

B Single cell from the same section X 1380. The residual mass of the disintegrated chloroplasts is shown collected near the central portion of the cell. It contains large numbers of faintly stained bodies resembling minute bacteria.

C Stain of a second subplant of a culture of coccus, No. 38, isolated from a mosaic navy bean plant. The organism resembles the Rickettsiae. Giemsa stain. x 1380.

D-J Stages in the cyclogenic development of the organism isolated in April 1928 from young seeds of Refugee bean plants affected with rugose mosaic. Beginning with the branching and curiously shaped cells shown in D and E, the organism passed through a progressive series of polymorphic changes which terminated in the form shown in J when it could no longer be cultured in subplants. At this stage the cultures contained large numbers of very small, coccus-like organisms, coherent in viscous masses. The various stages of development were produced in the bean pod juice to which living tissue was added. Cover slip smears stained with Giemsa stain. Photographed with 2 mm. apochromatic oil immersion objective, N. A. 1.40, periplan ocular 15 X. Wratten M plate, Wratten B screen. x 1380.